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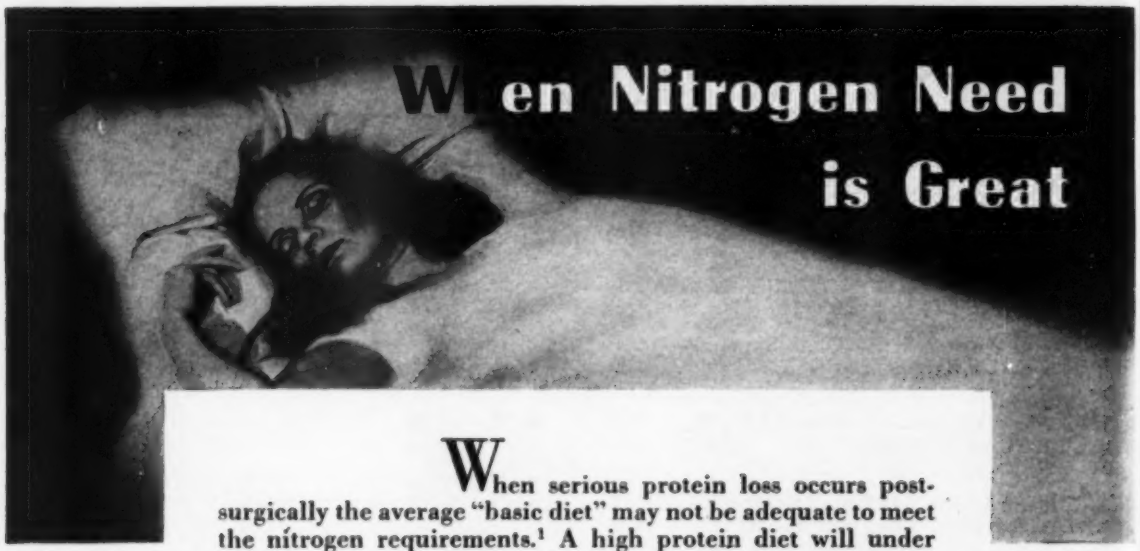

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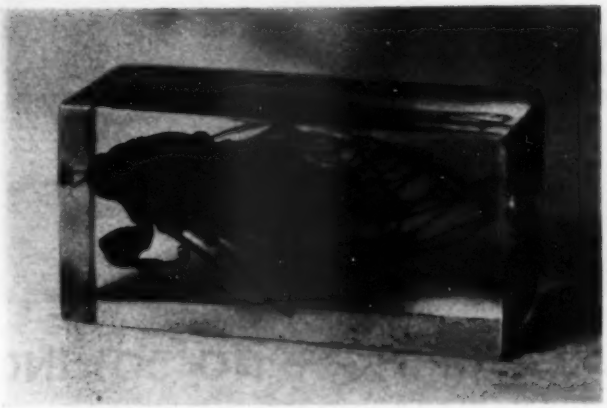

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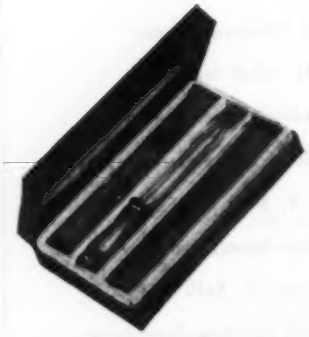

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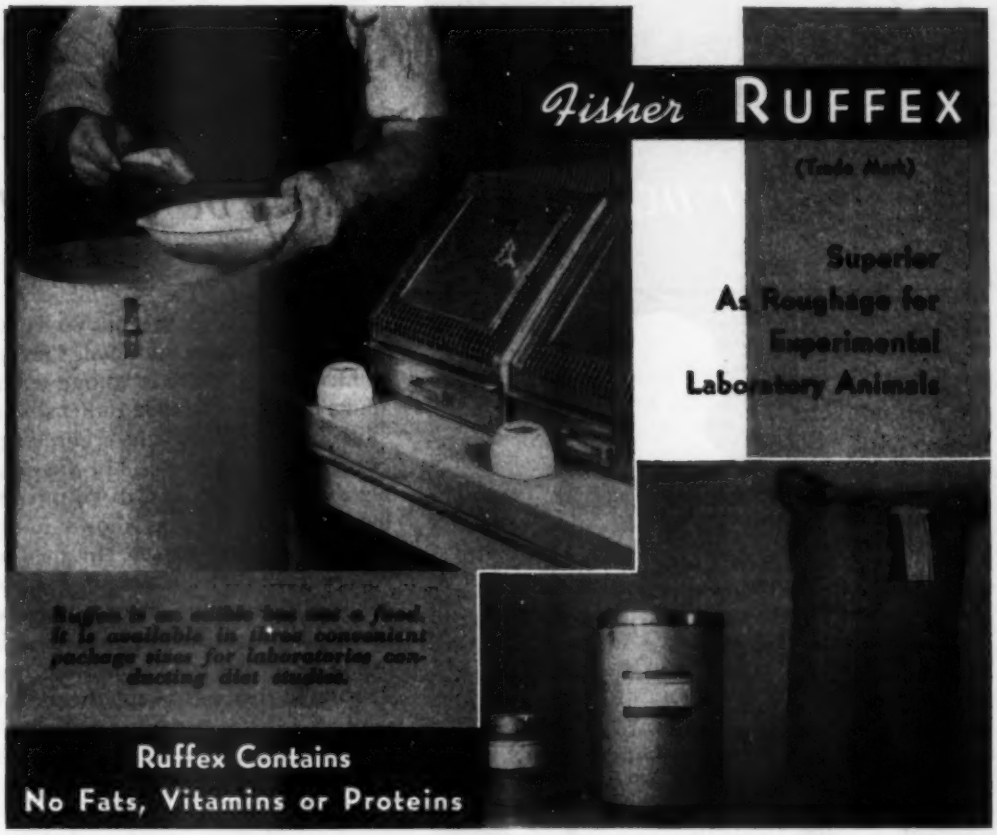

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ARCHIVES OF PATHOLOGY

VOLUME 40 SEPTEMBER 1945 NUMBER 3

PATHOLOGY OF LYMPHOCYTIC CHORIOMENINGITIS IN MICE

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In our original report [1] the pathologic studies were limited to the central nervous systems of mice and monkeys. Later reports of ours on monkeys [2] and guinea pigs [3] and of Rivers and Scott,[4] Traub,[5] Findlay and co-workers,[6] Lépine and colleagues,[7] Kasahara and co-workers [8] and Perrin and Steinhaus [9] have described the visceral as well as the cerebral lesions in mice, guinea pigs, rats and monkeys, and autopsies on 3 human beings have been reported by Machella and co-workers [10] and Smadel and co-workers.[11]

The present report deals primarily with mice in which visceral and cerebral lesions were observed following inoculations with various strains of virus from 1933 to 1945. It includes a series of mice inoculated originally in connection with the study of Perrin and Steinhaus [9] on Humphreys' [12] virus. Owing to exigencies of the United States Public Health Service, this material was turned over to us for study. As Humphreys' strain had shown certain differences in guinea pigs, it is hereafter discussed separately as the Humphreys strain. The material inoculated with it comprised 47 mice, 24 inoculated intracerebrally and 23 intraperitoneally, together with 24 controls inoculated in the same way with supposedly uninfected material. The other material comprised 8 mice on which reasonably complete autopsies were done from the 1934 series, 24 from the 1939 series, 24 from a series inoculated with virus 1565 in 1943, 57 from a series inoculated with virus 1650 in 1945, and scattering animals from series inoculated with other virus strains, as well as some 700 brains.

From 1933 to 1939 inoculations were almost entirely intracerebral, and gross reactions sufficiently impressive to call for microscopic study were encountered only in some 12 of 500 mice. The histologic reactions encountered in this series agree well with what has been observed since.

In the spring of 1939, several fresh virus strains, notably 945 and 947, were studied, and gross visceral lesions were more often observed. This tendency of freshly isolated strains to produce visceral lesions has been observed repeatedly since then. The appearance of gross lesions of the viscera led to further histologic study and to the more frequent use of noncerebral routes of inoculation.

The most frequent gross abnormalities seen were engorgement and enlargement of the spleen to perhaps twofold or threefold its normal size, pallor or yellowish discoloration of the liver and cloudy swelling of the kidney. Less often there were more or less profuse pleural exudates of clear or sometimes turbid or blood-tinged fluid, at times exceeding 1 cc. Similar peritoneal exudates, subcutaneous edema and grossly evident cervical adenopathy were infrequently noted. Pulmonary atelectasis, congestion and focal hemorrhage were sometimes noted, especially in the presence of pleural exudate.

With the Humphreys strain, a lesser splenic enlargement, usually less than twofold, and occasional lymphadenopthy were the only pathologic conditions noted.

From the Pathology Laboratory and the Division of Infectious Diseases, National Institute of Health.

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Findlay and Stern [6a] and Traub [5a] also noted serous exudates; the former were the first to note the rose to yellow coloration of the liver.

HISTOLOGIC EXAMINATION

Brain.—Of a total of some 760 brains studied since the isolation of the first strain of this virus in 1933, readily accessible records as to duration of infection after inoculation and route of inoculation were available for 209 mice studied between 1939 and 1945. Of these, 149 received intracerebral inoculation alone, 16 intracerebral combined with some other route of inoculation, and 44 inoculations other than intracerebral.

The 165 intracerebrally inoculated mice died or were killed as follows: 9 on the third, 7 on the fourth, 6 on the fifth, 43 on the sixth, 66 on the seventh, 16 on the eighth, 12 on the ninth, 3 on the tenth and 1 each on the twelfth, thirteenth and sixteenth days. The 44 infected mice not inoculated intracerebrally perished—6 on the third, 5 on the fourth, 3 on the fifth, 9 on the sixth, 13 on the seventh, 2 each on the eighth, ninth and twentieth and 1 each on the eleventh and twenty-eighth days.

On the third to fifth day intracerebrally inoculated mice presented a relatively slight lymphocytic infiltration of the meninges, more on the base than dorsally or in major fissures, and more often focal than diffuse. Choroid plexus appeared in 87 locations and showed no lesions in 84 and very slight to slight lymphocytic infiltration in 3, all in the third ventricle. In the parenchyma there were no lesions except the inoculation wounds. On the sixth to ninth day meningeal infiltration reached its greatest average intensity. It was greatest on the base and in major fissures, particularly between the brain stem and the overlying hippocampal and occipital cortex. On the sixth to eighth day, the infiltration consisted chiefly of lymphocytes, but in perhaps 10 or 15 per cent of the mice included lesser numbers of macrophages [13] or intact or fragmenting polymorphonuclear leukocytes. Lymphocytic infiltration of choroid plexus was seen occasionally on the fourth and fifth days; in slight to moderate grade it appeared regularly in the six day mice, and reached its maximum on the seventh to ninth days. On the average, involvement was greater in the choroid plexuses of the third and fourth ventricles than in those of the lateral ventricles. Proximity to the inoculation wound, which was most often found in the thalamus, might account for the relative increase in the infiltration of the plexus of the third ventricle but not for that of the plexus of the fourth ventricle. Inoculation wounds were encountered in the routine sections of 61 mice, involving the thalamus in 37 mice, the parietal cortex in 17, the hippocampus in 11, the frontal cortex in 6, the corpus striatum in 3, the midbrain in 2, the occipital cortex in 1 and the third ventricle in 1. Further, plexal infiltration was relatively greater in the third and fourth ventricles than in the lateral ones in mice not inoculated intracerebrally. Intraventricular exudates were first visible in mice dead on the sixth day. They consisted chiefly of lymphocytes and appeared with about equal frequency and intensity in the lateral, third and fourth ventricles in 20 to 25 per cent of the mice. On the seventh day predominantly lymphocytic exudates were present in the ventricles in about a third of the mice. Sometimes macrophages, desquamated ependymal cells, polymorphonuclear leukocytes or red corpuscles and perhaps fibrin appeared in the exudate. On the eighth and ninth days lymphocytic exudates were present in the ventricles in nearly half of the mice, but other cellular elements were seldom seen. During the period of greatest meningeal, plexal and ventricular involvement, lymphocytic infiltration was not infrequently seen in the sheaths of perforating submeningeal and subependymal vessels, and sometimes distinct edema of the corona radiata next the lateral ventricles was observed. This perivasculitis was generally of slight, sometimes of moderate, extent and was often absent even on the seventh to ninth days. Occasional foci of cellular gliosis were observed in a few mice.

After the ninth day, meningeal, plexal, ventricular and parenchymal involvement all diminished.

Mice inoculated by some other route in addition to the intracerebral showed a roughly similar grade of reaction, not definitely less than that with intracerebral inoculation alone. Mice inoculated by the intraperitoneal, the intranasal, the intravenous or the subcutaneous route without concomitant intracerebral injection of the virus showed sparse focal infiltrations of the meninges in isolated mice killed on the third to fifth days, with similarly infrequent foci of lymphocytic infiltration of the choroid plexuses of the third and fourth ventricles. Of 54 plexal locations, 4 showed foci of infiltration. Meningeal infiltration remained focal, but became more frequent on the sixth and seventh days, and 16 of 40 plexal sections showed slight, moderate or even marked lymphocytic infiltration in animals infected with virus 1650. Plexal infiltration was absent in 10 mice infected with other virus strains. Meningeal infiltration reached an intensity comparable to that in intracerebrally inoculated animals on the eighth and ninth days, and perhaps thereafter there was a longer persistence of meningeal and plexal infiltration. Intraventricular exudates were strikingly infrequent, but the ependymitis of the lateral ventricles so often observed in intracerebrally inoculated mice was also as frequent and as pronounced as early as the third day. It seems indicated that the ventricular exudates are perhaps more the result of introducing foreign material into the brain than a result specifically of the virus infection.

In the Humphreys strain series, ependymitis of this type was almost regularly present in all the intracerebrally inoculated mice, both virus-inoculated and controls, and was nearly as frequent in the intraperitoneally infected mice and controls. In this series of mice from Hamilton, Montana, the frequency of this particular lesion was greater than has usually been observed in any series studied here in the Washington, D. C., area.

In the Humphreys strain series, meningeal and cerebral reactions first appeared on the sixth day after intracerebral inoculation. After intraperitoneal inoculation, slight meningeal infiltration was evident on the sixth day, but slight intracerebral infiltration of vessel sheaths appeared first on the eighth day. In both series vessels mantled by lymphocytes were most conspicuous from the twelfth to the twenty-second day. They were more prominent with intracerebral inoculation. Meningeal and plexal infiltration appeared earlier, reached a greater intensity and persisted longer after intracerebral inoculation. In the parenchyma, especially in the caudate and lenticular nuclei, occasional foci of cellular gliosis were seen. They were most numerous about

13. We have adopted Metchnikoff's term "macrophage" to denote the potentially or actually phagocytic tissue wandering cell variously designated by such terms as "large mononuclear cell," "clasmatocyte," "histiocyte" and "polyblast."

three weeks after inoculation. They were less frequent after intraperitoneal inoculation.

Besides the ependymitis, which has been noted as occurring in control mice, the latter series included a few mice with well marked lymphocytic infiltration of the meninges and the choroid plexus, especially in those receiving intracerebral injections. Usually meningeal and plexal lesions were absent in the control series. Since such lesions usually have not been observed in the brains of mice from other experimental studies in the Washington, D. C., or Bethesda, Md., laboratories of the National Institute of Health, it appears probable that spontaneous infections, such as were reported by Traub,[5a] may have been present at that time in the Rocky Mountain Laboratory, where Steinhaus was working. Shortly after the time when Steinhaus collected his material from mice and guinea pigs for pathologic study, spontaneous infections with this virus were actually demonstrated in the animal stock in that laboratory.

Our 1934 report[1] noted the predominantly lymphocytic character of the meningeal exudate, with the presence of fewer macrophages and polymorphonuclear leukocytes; the greater infiltration on the base, in major fissures and around the brain stem beneath the overhanging cortex; the frequent but inconstant, comparatively slight and usually purely lymphocytic plexal infiltration, which was greater in the third and fourth than in the lateral ventricles. Rivers and Scott[4] reported similar observations in mice, and they noted increase in infiltration of vessel sheaths in later stages. Traub[5a] also reported essentially identical observations with regard to meningoplexal infiltration and further noted exudates of round cells in the ventricles, ependymal infiltration and subependymal gliosis. Essentially similar changes in mice were recorded by Findlay, Alcock and Stern[6b] and, in greater detail, by Findlay and Stern.[6a] They stressed ependymal lesions less and noted less meningeal exudate on the spinal cord. Also the observations of Lépine and Sautter[7a] and of Lépine, Kreis and Sautter[7b] were essentially similar. The latter group noted that the meningochoroiditis appeared about the sixth day in mice inoculated intracerebrally. Kasahara, Hamano and Yamada[8b] encountered meningochoroiditis of the same type and further noted the presence of congestion and focal hemorrhage in the choroid plexus, as well as lymphocytic infiltration. Ependymitis was fairly prominent in their series.

In guinea pigs meningeal and plexal infiltration have been noted as less in intensity and frequency than in mice by Rivers and Scott,[4] Traub,[5a] Kasahara and co-workers[8] and Lillie and Armstrong.[3] Findlay Alcock and Stern[6b] stated that the process was similar to that in mice, while Perrin and Steinhaus[9] reported infrequent meningeal and no plexal involvement. In our series meningeal reaction was constantly present from the sixth to the twenty-seventh day.[3]

Findlay and Stern[6a] observed a similar process in rats but stated that the exudate contained a greater proportion of polymorphonuclear leukocytes than was noted in other species. Kasahara and co-workers[8] stated that the reaction in rats was less marked than that in mice.

Monkeys have presented a more variable picture. Not only have the reports of the several observers shown variations but individual series of animals have revealed considerable differences. In our series[2] the plexal lesion varied from a focal, purely lymphocytic infiltration to a diffuse mixed infiltration in which lymphocytes, plasma cells and macrophages took part, with congestion and edema of plexal villi and intraventricular exudates of lymphocytes, macrophages, choroidal epithelium, few leukocytes, serum, fibrin and erythrocytes. Meningeal infiltration was moderate, chiefly lymphocytic and fairly general over the brain and spinal cord. Intracerebral infiltration of vessel sheaths was infrequent and focal gliosis rare. Findlay, Alcock and Stern[6b] stated that the general reaction in monkeys was less than that in mice, but later Findlay and Stern[6a] described a variation in reaction for Macaca mulatta and Macaca irus similar to that in our series. The significance of the very small granules in the cytoplasm of mononuclear cells in the ventricular exudate which they described remains uncertain. Kasahara, Hamano and Yamada[8b] noted lesions in rhesus monkeys similar to those in mice.

In man, Machella, Weinberger and Lippincott[10] reported meningeal fibroblastic proliferation, lymphocytic infiltration, focal hemorrhage and focal macrophage infiltration, the latter cells containing blood pigment. This patient died on the thirtieth day of illness. The meningeal exudate was more profuse on the base and around the brain stem, and here also there were patches of organizing fibrin. The ependyma presented much desquamation, perivascular lymphocytic infiltration and subependymal gliosis. Otherwise the parenchyma showed no significant lesions. In the choroid plexuses of the lateral ventricles there were irregular dense "inflammatory cell" infiltration, interstitial hemorrhage and necrosis of tips of choroidal villi. An intraventricular exudate included fibrin, erythrocytes, necrotic choroidal epithelium, macrophages and "inflammatory cells". Virus was not recovered. In 1 of the 2 human cases reported by Smadel, Green, Paltauf and Gonzales[11] there was a subdural hematoma as well as perivascular lymphocytic infiltrations of the meninges, the brain and other unspecified organs.

Spinal Cord, Meninges and Ganglions.—Meningeal lymphocytic infiltration was present only in 3 of 16 mice. The 13 spinal ganglions found in the 32 levels of cord studied presented no lesions; the levels examined in this series were usually low.

In the Humphreys strain series, meningeal lymphocytic infiltration appeared first at the sixth day and was present in 24 of 27 mice that were killed twelve to forty-four days after their inoculation. Both frequency and intensity of infiltration were somewhat greater after intracerebral than after intraperitoneal inoculation. Within the spinal cord, occasional foci of cellular gliosis were seen in 4 mice, lymphocytic infiltration of vessel sheaths of moderate grade was seen in 8 mice, and similar infiltration was noted about one or two vessels only in 4 more. In the two levels of spinal column sectioned in each of 47 mice, 68 spinal ganglions were encountered. Lymphocytic infiltration of either root zone or ganglion cell areas was noted in 11 ganglions from 7 mice.

Lymphocytic infiltration of meninges and of one ganglion was seen in 1 of 24 controls.

Rivers and Scott,[4] Traub[5] and Findlay and Stern[6a] noted lymphocytic infiltration of the spinal meninges in mice, which was usually of lower grade than that over the brain.

In guinea pigs Perrin and Steinhaus[9] observed no lesions of spinal cord, meninges or ganglions. In our guinea pigs,[3] the cord was not studied, but numbers of sympathetic ganglions from cervical and abdominal regions were normal, and in one of three ganglions containing chromaffin cells there was focal lymphocytic infiltration. In monkeys we observed a relatively slight meningeal lymphocytic infiltration of the cord, frequent focal lymphocytic infiltration or proliferation of sheath cells in root ganglions and infrequent lesions within the

cord proper. The last included lymphocytic infiltration of vessel sheaths, focal hemorrhage and focal cellular gliosis. Meningeal lymphocytic infiltration was also relatively sparser in the patient whose case was reported by Machella, Weinberger and Lippincott.[10]

Skeletal Muscle.—Specimens of cross-striated muscle from 80 mice were studied microscopically. Fifteen specimens were vertebral, 17 cervical, 68 crural, 4 pelvic and 2 diaphragmatic. Occasional foci of interstitial or perivascular lymphocytic infiltration were found in the leg muscles of 8 mice. Four of these had been inoculated intracerebrally and the other 4 intraperitoneally. In 2 mice there was severe pelvic cellulitis with slight to moderate lymphocytic infiltration of the adjacent striated musculature. In another instance there was cervical cellulitis with slight lymphocytic infiltration of adjacent muscle. These last 3 mice had been inoculated, 2 intraperitoneally and 1 intravenously, and survived twenty, twenty and twenty-eight days, respectively.

In the Humphreys strain series slight to moderate focal lymphocytic infiltration was observed in the cervical muscles in 3 of 26 mice and in the vertebral musculature in 4 of 47. No lesions appeared in 20 and 24 control specimens, respectively.

About a third of our guinea pigs [3] showed occasional foci of perivascular lymphocytic infiltration or of concentric vascular endothelial proliferation, and similar foci occurred in muscle in 1 monkey. Focal necroses such as Perrin and Steinhaus [9] observed in guinea pigs occurred also in controls and were not considered significant.

Submaxillary Glands.—Submaxillary glands from 75 mice were studied, 29 of which had been inoculated by the intraperitoneal route alone, 34 by the intracerebral route alone and 12 by various other single or mixed or unknown routes. After intracerebral inoculation a few foci of lymphocytic infiltration were seen in 2 of 7 mice killed on the fourth day, more extensive lymphocytic infiltration, chiefly interlobular, was evident in 5 of 8 mice killed on the sixth day, and on the seventh day lesions were almost regularly present. Only 3 of the 27 intraperitoneally inoculated mice which died from the third to the seventh day showed focal or diffuse infiltration. More profuse interlobular and interstitial lymphocytic infiltration was seen in 7 of 10 mice which died eight to twenty-eight days after inoculation. With more profuse lymphocytic infiltration, serous exudate, patches of fibrin, foci of hemorrhage and numbers of plasma cells also appeared. Again interlobular and periglandular tissues were more severely involved.

It appeared indicated that this sialadenitis was at least in part secondary to cervical lymphadenitis, which was evident as early as five days after intracerebral inoculation and seven days after intraperitoneal. In a number of instances the infiltration of the salivary glands was limited to the neighborhood of involved lymph nodes.

With the Humphreys virus, a slight to moderate, occasionally marked lymphocytic infiltration was noted about ducts and vessels and interstitially in 12 of 23 mice inoculated intracerebrally and in 7 of 23 inoculated intraperitoneally. It was absent in both series of controls (24 mice). Frequency and intensity were perhaps greatest at six to ten days.

Cervical fascia was infrequently and slightly involved, though cervical lymphadenitis was present.

Interstitial lymphocytic infiltration was noted in 1 of our 17 monkeys [2] and 3 of our 12 guinea pigs.[3] Traub [5b] noted no lesions, while Perrin and Steinhaus [9] encountered quite frequent periductal and interstitial lymphocytic infiltration, probably more often and of greater extent in infected than in control guinea pigs.

Thyroid and Parathyroid Glands.—Thyroid glands from 8 mice which died six to twenty days after inoculation were studied. In the last one of these mice only was there focal interstitial lymphocytic infiltration. This mouse also presented the most severe interstitial sialadenitis of any studied.

In the Humphreys virus series 21 inoculated and 18 control mice showed no lesions. They were killed one to forty-four days after the injections were made. Three parathyroid glands were also without evidence of infection.

We saw no lesions of the thyroid gland in monkeys [2] or in most of the guinea pigs.[3] In 2 of the 14 guinea pigs there were interstitial edema and lymphocytic infiltration, respectively. Perrin and Steinhaus [9] saw no lesions in their guinea pigs.

The parathyroid glands of the guinea pigs in our series [3] and in that of Perrin and Steinhaus were normal.[9] As to monkeys,[2] we noted focal necrosis in 1 animal, focal lymphocytic infiltration in 3 others and no lesions in 5.

Trachea.—Thoracic and cervical levels of the trachea were studied in 27 and 12 mice, respectively, and both in 2 mice, so that a total of 37 mice was represented. Slight to moderate lymphocytic infiltration was noted in the mucosa in 11, chiefly after the seventh day. In 2 mice there were suppurating ulcers at the thoracic level, and in a third, purulent tracheitis.

In the Humphreys strain material, focal lymphocytic infiltration appeared in the laryngeal mucosa in 18 of 23 inoculated mice and in 4 of 6 controls. The significance of this infiltration appears dubious.

In the trachea, however, only 2 of 29 inoculated mice and 1 of 20 controls showed irregular mucosal lymphocytic infiltration.

Focal lymphocytic infiltration of the mucosa of the larynx, the trachea and the bronchi has been noted also in a minority of our monkeys,[2] more often in our guinea pigs.[3]

Extensive necrotizing pharyngitis was present in 1 of 2 human cases of lymphocytic choriomeningitis reported by Smadel, Green, Paltauf and Gonzales.[11]

Lungs, Pleurae and Mediastinum.—A histologic study of the lungs of 110 mice was made. Associated with the presence of gross fluid exudates in the pleural cavity, partial atelectasis was often evident microscopically, and was seen also in other mice in which the gross observation was lacking. Pleural exudates seemed more frequent in mice inoculated by the intraperitoneal and subcutaneous routes than in those infected intracerebrally or intranasally.

Focal perivascular lymphocytic infiltration occurred in intraperitoneally infected mice as early as the third or fourth day and, in less grade and frequency, after intracerebral inoculation from the fourth day on. Altogether it was present in 45 mice and was more frequent after nine days. Congestion and focal alveolar hemorrhage appeared in 26 mice dying from the third to the ninth day, while serous alveolar and bronchial exudate was present in 19, chiefly from the seventh to the ninth day.

Sparse focal pneumonia was observed in 8 or 9 mice. In appearance it was similar to that seen in mice dying of other conditions. It occurred in mice dying six, seven, eight (3 mice), nine, ten and unknown numbers of days after inoculation.

Exudative mediastinal or pulmonary pleurisy was noted in 26 mice. In this process there were swelling and desquamation of mesothelial cells, exudation of serum, fibrin, lymphocytes, red corpuscles, fewer macrophages and plasma cells and karyorrhexis and necrosis of

exudate. Earlier exudates consisted chiefly of lymphocytes. These exudates were more frequent and more profuse on the mediastinal than on the pulmonary pleura.

Subpleural infiltration by lymphocytes, at times plasma cells, lymphocytes, serum and fibrin as well, and sometimes focal hemorrhage were observed in the mediastinal fatty tissues of 74 of 95 mice and in the pulmonary pleurae of only 9 of the 110 mice. In general, exudates appeared earlier and were more profuse with intraperitoneal than with intracerebral inoculation, and the mediastinal infiltration more often included serum, plasma cells, macrophages and hemorrhage.

Exudates were most common from the sixth to the eighth day. Patches of fairly dense infiltration by lymphocytes alone appeared in the mediastinal pleura in some animals as early as the third and fourth days, and such infiltrations occurred also in other mice. However, the presence of gross or microscopic pleural exudate was nearly always associated with cellular infiltration of the mediastinal or the visceral pleura.

Mediastinal lymph nodes were found in sections from 48 of these mice. In 23 animals killed on the third to fifth day, there were usually no significant lesions. On the sixth and seventh days the mice inoculated by other than the intracerebral route showed lesions in most instances. In some nodes only accumulation of nuclear debris in the follicular lymph clefts and phagocytes were noted. In others there were areas of edema and depletion of cells in the pulp, sometimes accompanied by exudation of fibrin and karyorrhexis or even diffuse coagulation necrosis. In some mice, predominantly follicular reactions were present in some nodes, and the pulpal lesion in others.

Accompanying this lymphadenitis there was constantly more or less mediastinal infiltration with edema and usually pleural exudation.

In intracerebrally inoculated mice similar but less pronounced and less frequent reactions were present, consistent with the lesser frequence and severity of mediastinal infiltrations and pleural exudates.

In 5 of 7 mice surviving eight to twenty days, reaction was absent; 1 mouse showed edema of pulp and sinuses, and the seventh moderate phagocytosis of nuclear debris in the follicles.

In the Humphreys strain series, there were occasional isolated alveolar hemorrhages unassociated with other lesions in 5 mice, 2 inoculated ones and 3 controls. Only 1 mouse showed congestion, hemorrhage and serous exudate in the alveoli. Perivascular lymphocytic infiltration of variable grade occurred in 24 of the 46 mice, affecting 22 of the 26 that were killed from the twelfth to the forty-fourth day. It was most pronounced on the eighteenth to twenty-sixth days. Aside from 1 mouse with pneumonia, slight perivascular lymphocytic infiltration was seen in 2 of the 24 controls. The route of inoculation, whether intracerebral or intraperitoneal, was without influence.

Infiltration of the visceral pleura by lymphocytes was noted in 9 infected and 2 control mice, all of which presented intrapulmonary infiltrations also.

Patches of dense lymphocytic infiltration were usually noted in mediastinal fat, perhaps to a greater extent and amount in intraperitoneally than in intracerebrally inoculated mice and more in these than in controls, but the differences were not great. In 2 mice there were, respectively, patches of fibrin exudation and of edema and infiltration by macrophages and lymphocytes.

Mediastinal lymph nodes were present in 28 inoculated and 11 control mice. Follicular swelling with dilated lymph clefts and free and phagocytosed nuclear debris was noted in 5 or 6 of the inoculated mice, but no significant lesions were found in the pulp.

Pulmonary lesions varying in extent (and concept) from perivascular lymphocytic infiltration to interstitial pneumonia have been reported in mice by Rivers and Scott,[4] Traub,[5a] Findlay and Stern [6a] and, probably, Lépine and Sautter.[7a] Findlay and Stern stated that interstitial bronchopneumonia was rarer after intracerebral than after intraperitoneal inoculation. Similar lesions were observed in guinea pigs by Rivers and Scott,[4] Traub [5a] Findlay and Stern,[6a] Kasahara, Hamano and Yamada,[8b] Lillie and Armstrong [3] and Perrin and Steinhaus.[9] Rivers and Scott stressed the interstitial pneumonic character of the lesion, Traub characterized it as of "virus type," we rather discounted the lesions as not greatly in excess of the usual findings in uninoculated guinea pigs, and Perrin and Steinhaus found equal extent and frequency of infiltration in their control series. Traub noted pulmonary edema, which was paralleled in the present series of mice and resembled that described in some of our rhesus monkeys. In the latter species, in our series, congestion, edema and perivascular lymphocytic infiltration were apparently the essential features. Findlay and Stern observed only lymphocytic infiltration in this species. Focal pneumonia was present in both of the human cases reported by Smadal, Green, Paltauf and Gonzales.[11] In 1 case the cells of the alveolar exudate varied from chiefly polymorphonuclear leukocytes to mononuclear cells exclusively from area to area. In the other the grossly hemorrhagic pneumonia was characterized microscopically by infiltration of septums by mononuclear cells, proliferation of "alveolar cells" and intra-alveolar exudation of fibrin, erythrocytes and large pale mononuclear cells. The virus was isolated from both patients. Rats showed a process similar to that in guinea pigs, according to Findlay and Stern [6a] and Kasahara, Hamano and Yamada.[8b]

Thymus.—Sections were made from the thymuses of 40 mice infected with virus 1650, 20 inoculated intraperitoneally, 20 intracerebrally, and from those of 34 of the Humphreys strain series.

The essential thymic lesion was karyorrhexis and necrosis of cortical lymphocytes. This was accompanied by dilatation of lymph spaces in both the cortex and the medulla, accumulation of nuclear debris within these spaces, swelling of the littoral cells lining them and phagocytosis of nuclear remnants by the littoral cells. With the 1650 virus, this process appeared on the fourth day after intraperitoneal inoculation and on the sixth or seventh after intracerebral. Cell necrosis soon resulted in great depletion of the cortex, and the concomitant swelling of littoral cells produced an "epithelioid" replacement.

With the Humphreys virus, the same process was evident in 18 of 19 mice killed on the sixth to eighteenth days and absent in 7 killed on the first, second and fourth days and in 8 killed on the twenty-second to forty-fourth days. While the aforementioned difference in time of appearance of the lesion was not repeated in this series, the process seemed more severe in the intraperitoneally inoculated series.

The 23 control mice of the Humphreys strain series showed no thymic lesions.

We found no lesions in 4 monkeys [2] or in 8 of 9 guinea pigs.[3] One guinea pig had cortical hyperplasia of the thymus with phagocytic cortical littoral cells ingesting nuclear debris. Perrin and Steinhaus [9] observed in guinea pigs acute cortical degeneration and necrosis resembling that described in mice (see a foregoing paragraph).

Heart.—In 64 of 105 mice there were foci of infiltration, showing chiefly lymphocytes but including

macrophages or fibroblasts in some mice. Infiltration was more common in the epicardium and in the atriums but occurred also in the myocardium and the endocardium and in the ventricles. It was more frequent and more profuse after the seventh day and somewhat less after intracerebral inoculation.

A ventricular infarct was present in 1 mouse, aortic endocarditis in 1 and focal myocardial calcification in 2; in 40 mice there were no lesions.

With the Humphreys strain, a similar lymphocytic infiltration of similar distribution was recorded in 28 of 46 mice, and the 24 controls showed no lesions.

Traub [6a] observed similar lesions in guinea pigs. We noted vascular endothelial proliferation, focal fibroblastic proliferation and lymphocytic infiltration of more marked grade than in mice in all of our guinea pigs,[3] in muscle, endocardium and epicardium. Macrophages, polymorphonuclear leukocytes and plasma cells were sometimes mingled with the lymphocytes. Perrin and Steinhaus [9] reported similar lesions in both infected and "control" animals.

Findlay and Stern [6a] observed small areas of lymphocytic infiltration of heart muscle in monkeys, and similar foci were present in 10 of our 23 animals.[2]

Gastrointestinal Tract.—The esophagus was examined in 56 mice—at the laryngeal level in 8, at the midthoracic level in 49 and at the cardiac end in 5. One mouse had an extensive infiltrative and proliferative reaction in mucosa, muscularis and serosa, associated with an apparent tear in the mucosa. This was at the thoracic level. Otherwise, focal lymphocytic infiltration of the mucosa was noted in 4 mice, involving the cervical level in all and the thoracic in 1 of them. A serosal lymphocytic infiltration extended into the muscularis at the cardia in 1 mouse.

Sections of the stomachs of 52 mice were made, and more or less pronounced peritoneal reactions were present in 36 of these. Of these 36, 10 showed lymphocytic infiltrations in muscularis, submucosa or mucosa or perhaps in two or more layers. Such infiltrations usually appeared in the antrum or the fundus. A small granulating ucler was seen in the proventriculus in 1 mouse and focal necrosis of the mucosa of the fundus in 2 others.

Sections of 228 levels of the small intestines of 81 mice were studied. Usually these showed no lesions of mucosa, submucosa or muscle. Lymphocytic infiltration of Brunner glands was seen in the duodenum in 2 mice, ulcerative enteritis in 1 and clumps of phagocytes in the villi in another. Serosal reactions, usually mesenteric, were noted in several. Lymphoid follicles were noted in 13 mice, showing hyperplasia, alone or in combination with slight or marked accumulation of nuclear debris in the follicular lymph clefts. Some phagocytosis was apparent in the latter.

Sections of 70 levels of the colons of 31 mice were studied. Increasing activity of mucous glands toward the rectum was noted. Mucoepithelial exudate was noted in the lumen of the rectum in 8 of 17 mice and in that of the colon in 9 of 28 and was generally more profuse in the rectum. Lymphoid follicles exhibiting hyperplasia, karyorrhexis and phagocytosis were observed in 5 mice. Otherwise, occasional foci of lymphocytic infiltration were seen in the serosa and the muscularis.

In the Humphreys strain series, focal lymphocytic infiltration of esophageal mucosa or muscularis was seen in 6 of 44 inoculated mice and in none of 22 controls. Five of the animals with cellular infiltration had been inoculated intraperitoneally and 1 intracerebrally. The duration after infection in these 6 mice ranged from eight to twenty-six days.

Similarly irregular lymphocytic infiltration of glossal and of pharyngeal mucosa was noted in 9 of 33 inoculated and 1 of 11 control mice. It was usually slight or focal in character.

In the stomach, lymphocytic infiltration of submucosa, muscularis or mucosa was noted in the fundus or, less often, the proventriculus in 12 of 22 mice inoculated intraperitoneally and in 8 of 19 inoculated intracerebrally. It also was noted in 7 of 21 controls. The increase in the intraperitoneal series was at most moderate, in the intracerebral series slight, over the controls.

The small intestine was studied at 120 levels in 41 mice. In 1 mouse there was focal leukocytic infiltration of villi, in another seropurulent exudate in the crypts, in another congestion and focal mucosal hemorrhage. In 2 there was lymphocytic infiltration of the serosa, and in 2 lymphoid follicles showed accumulation of nuclear debris in follicular lymph clefts with phagocytosis. At 113 levels there were no lesions. In 22 control mice 63 levels showed no lesions; 1 control showed slight, 1 moderate, phagocytosis in a lymphoid follicle, with concomitant slight purulent exudate in crypts.

The colon of 1 mouse presented a purulent ulcer, that of another pus-filled glands and that of a third lymphocytic infiltration of the serosa; in the colons of the remaining 7 mice there were no lesions beyond moderate secretion of mucus. A total of 21 sections of colon were studied.

Similarly, significant mucosal lesions were lacking in guinea pigs in both our series [3] and that of Perrin and Steinhaus [9] and in our monkeys.[2] Again lymphoid follicle reactions like those in lymph node follicles appeared in our guinea pigs and monkeys.

Liver.—Histologic examination was made of the livers of 112 mice. With 16 of the earlier livers, no fat stains were done, but in 9 of them the cytoplasm of the liver cells showed fine foamy reticulation. In sections of 4 livers that were stained in 1939 by the Herxheimer technic, no fatty changes were evident, but those stained in 1943 by both the Herxheimer and the supersaturated 60 per cent isopropanol technic showed that several livers were nonfatty by the first and fatty by the second method. The remainder of the livers presented deposition of fine fat droplets in the cytoplasm of liver cells, sometimes only in the periphery, more often throughout the cytoplasm. While this fatty change was often diffuse in all parts of the lobules, it also occurred in patchy areas, more often midzonal in location, extending frequently to the portal areas and often also to the hepatic venules. Noteworthy in some livers was the occurrence of isolated heavily fat-laden liver cells in otherwise fat-free areas and conversely the presence of isolated fat-free cells among cells heavily laden with fine fat droplets.

Already on the third day, moderate irregular or diffuse fatty changes were present in liver cells. These changes became most severe on the seventh to ninth days and continued evident through the twelfth day. Two mice killed on the twentieth day had no fat in the liver by the Herxheimer method, and 1 killed on the twenty-eighth day showed nonvacuolated liver cells in paraffin sections.

Capillary congestion was prominent in mice killed on the third day but not in those killed thereafter. Occasionally on the fifth day, frequently on the sixth and seventh and sometimes also on the eighth and ninth days there were scattered, isolated coagulated necrotic liver

cells and small rounded foci of coagulation necrosis. The latter lesion seemed limited to early passage generations of the virus, but occurred with several strains. Both in multicellular foci and about isolated necrotic cells there was often a prominent proliferative reaction of rounded and fusiform cells with leptochromatic nuclei and broad cytoplasm. More frequent even were small rounded granulomas composed of similar epithelioid cells. A minority of these granulomas contained coagulated liver cells or a little centrally placed nuclear debris or perhaps a polymorphonuclear leukocyte or two or sometimes a few lymphocytes.

Sometimes associated with necrosis of liver cells, or perhaps apparently independent of it, there was hyaline, fibrinous or necrotic cellular thrombosis. This process was noted in 10 mice, necrosis of liver cells in 54 mice and granulomas in 40; in 41 mice there were none of these lesions. The last group included 23 of the 36 mice killed on the third to fifth days.

Patches of capillary dilatation and myelosis, with definite erythroblasts and megakaryocytes, were observed in 30 mice. This focal myelosis was relatively about half as frequent after intracerebral inoculation, whereas the cellular necroses and granulomas were equally frequent with cerebral and other routes of inoculation.

Likewise, periportal and sometimes interstitial lymphocytic infiltration was little influenced by route of inoculation. This infiltration was comparatively infrequent, sparse and slight before the seventh day and was most frequent and profuse in the mice surviving over ten days. The ratio absent: slight: moderate: marked was 16:6:1:0 at three to four days, 29:7:13:12 at five to seven days, 5:0:6:5 at eight to ten days and 0:0:0:5 at eleven to twenty-eight days.

With the Humphreys strain, material was saved for fat stains only from the mice killed from one to fourteen days after inoculation. Slight to moderate accumulation of fine fat droplets in liver cells in midzonal and perhaps periportal areas was evident as early as one and two days after inoculation. By the fourth day this process was fairly well marked and remained severe from the sixth to the tenth or twelfth day. It was definitely decreased on the fourteenth day. Again route of inoculation had little evident influence, the fatty degeneration being perhaps slightly greater after intracerebral inoculation.

Necrosis of isolated liver cells or of small foci of such cells was seen in only 7 of the 47 mice, 5 inoculated intracerebrally. Hyaline thrombi were rarely seen. One necrotic focus was seen in 1 control mouse and small purulent foci in another.

Intracapillary myelosis, as noted in a foregoing paragraph, occurred in 11 mice, with megakaryocytes in 5. In this series the frequency appeared greater after intracerebral inoculation.

The most prominent lesion in this series was perivascular, chiefly periportal lymphocytic infiltration. Again it became more prominent and more frequent in the second week than in the first and remained about as prominent to the end of the observation period of forty-five days.

Findlay, Alcock and Stern [6b] first noted a yellow or rose discoloration and fatty degeneration of the liver in mice dying with the usual neurologic syndrome. Findlay and Stern [6a] described infiltrations varying from a few cells to a leukemoid picture. Among the dominant lymphocytes were plasma cells, large mononuclears and a few polymorphonuclears. "Here and there the liver cells had undergone necrosis." Lépine and Sautter [7a] noted only focal infiltration, while Traub [5a] observed lymphocytes and mononuclear cells infiltrating about vessels frequently, regardless of the route of inoculation. Focal necrosis he observed only after intravenous inoculation. Rivers and Scott [4] noted a few small foci of necrosis.

In guinea pigs Traub observed focal accumulation of round cells, fatty vacuolation and large areas of necrosis. The last finding he discounted as probably an intercurrent lesion. Similar infiltration, fatty degeneration and small foci of necrosis, like those in other species, were reported by Findlay, Alcock and Stern, by Findlay and Stern, by us [3] and by Perrin and Steinhaus.[9] In the material of Findlay and Stern, focal necroses were "oxyphil"; in ours some were coagulative, while others were in process of replacement by proliferative reactions; the observations of Perrin and Steinhaus were similar to ours. Focal necrosis, fatty changes and perivascular lymphocytic infiltrations were also found in our monkeys and in those of Findlay, Alcock and Stern.

Pancreas.—The sections of pancreas studied represented 82 mice. In 26 mice there was more or less lymphocytic infiltration, often apparently primarily peritoneal and interlobular in location, sometimes distinctly periductal and perivascular, and in 8 mice definitely interstitial. Such reactions were present in 7 of 8 mice examined from the ninth day on, in 14 of 36 examined on the sixth to eighth and in 2 of 33 on the third to fifth days. They appeared later and less frequently after intracerebral inoculation. Interlobular edema was noted in 5 of these 26 mice, and in 1 animal whose pancreas was otherwise normal there was interlobular hemorrhage. These lesions were usually early, though 1 mouse dead on the twenty-eighth day presented congestion, edema and striking lymphocytic infiltration. Sometimes macrophages or plasma cells participated in the infiltration, but generally it was chiefly lymphocytic. In 1 mouse there were occasional hyaline or necrotic cellular thrombi in capillaries.

With the Humphreys strain, interlobular edema was observed in 10 of the 16 mice killed on the sixth to twelfth days. Interlobular lymphocytic infiltration of variable grade appeared in all 16 mice killed on the eighth to fourteenth days, in 8 of 12 killed on the eighteenth to twenty-sixth days, in 3 of 4 killed on the sixth day and in 1 of 3 killed on the forty-fourth day. From the twelfth to the twenty-sixth day this infiltration extended in about ducts and vessels and between acini. As a probable result of this interstitial infiltration, more or less pronounced focal dilatation and atrophy of acini were noted in 12 mice. In some of the later animals, this infiltration was quite irregular, dense in some areas, sparse or entirely absent in others.

None of the 8 mice killed on the first to fourth days showed any lesions. Of the 23 controls, 1 showed slight focal interlobular and perivascular lymphocytic infiltration.

As to monkeys, we [2] recorded irregular periductal and interstitial lymphocytic infiltration in only 2 of the 22 animals, and regarded acute processes in 3 more as of dubious significance. On reconsultation of the protocols, the acute processes in 2 of these seem to have been definitely intercurrent, but the one in the third, a diffuse interstitial lymphocytic infiltration, is to be regarded as significant. Findlay and Stern reported small foci of infiltrating lymphocytes.

In guinea pigs in Traub's [5a] series and ours [3] there were no significant lesions, and only 2 guinea pigs of the series observed by Perrin and Steinhaus [9] showed focal lymphocytic infiltration.

Peritoneum.—Histologically, peritoneal reactions are most often evident in the omentum and the mesentery, less often in the serosae, of the intra-abdominal viscera, such as the liver and the spleen. As in mice the pan-

creas lies in omental tissue, the serosa of this organ is often involved. The serosae of the bladder, the uterine tubes and the uterus and especially the broad ligaments often show involvement.

The commonest finding was lymphocytic infiltration of variable density, and while this sometimes appeared in uninfected mice, in this series from the sixth day on it was constantly present in the 26 mice infected by the intraperitoneal, subcutaneous and intravenous routes and appeared in 18 of the 37 mice inoculated by the intranasal, intracerebral and unspecified but probably intracerebral routes; 8 of 14 intraperitoneally inoculated mice showed lymphocytic infiltration.

Less often there were noted submesothelial serous exudate, mixed infiltrations in which macrophages or plasma cells and lymphocytes participated, mesothelial swelling, proliferation and desquamation, and serous to fibrinous exudates which sometimes contained numbers of lymphocytes, mesothelial cells, macrophages, red cells and intact or fragmenting polymorphonuclear leukocytes. The frequency of such exudates was greater after intraperitoneal inoculation.

Similar infiltration by lymphocytes was seen with the Humphreys strain. With intracerebral inoculation it was about four times as great in frequency and amount as in controls given intracerebral injections of supposedly normal animal tissue. With intraperitoneal inoculation the amount of this reaction was even greater, but the controls given intraperitoneal injections showed as much or more omental infiltration. Hence it would appear that the slightly greater reaction after inoculation by this route might be due to the intraperitoneal introduction of foreign material.

Traub [5a] noted serous pleuroperitonitis in 20 per cent of mice inoculated intraperitoneally. Mesothelial swelling and slight lymphocytic infiltration were observed microscopically. Findlay and Stern [6a] recorded similar gross findings and reported lymphocytes and large mononuclears as the chief cellular constituents of the exudate.

Focal lymphocytic infiltration of omentum and mesentery appeared in about a third of our guinea pigs,[3] and in some there was focal proliferation of fibroblasts or of mesothelium. Perrin and Steinhaus [9] recorded similar reactions in both infected and control guinea pigs after intraperitoneal injection.

Focal lymphocytic infiltration of omentum was present in 4 of our monkeys [2]; 1 had edema, hemorrhage and much diffuse and perivascular infiltration by lymphocytes and plasma cells. Two others presented helminthic abscesses of the mesocolon.

Altogether, it seems that in all series both the virus and the extraneous material injected intraperitoneally operated to produce reactions.

Spleen.—Sections were made from the spleens of 105 mice. The follicles were generally moderately active in earlier and later stages; they were quite hyperplastic on the sixth to eighth days. The phagocytic follicular littoral cells were often moderately prominent, especially on the sixth to eighth days, and less often there was slight to marked accumulation of nuclear fragments free in the lymph clefts and more often in the phagocytes. This karyorrhexis was more often noted on the sixth than on the seventh day. Occasionally it graded into frank karyorrhectic necrosis of follicular substance, but more often there were hemorrhagic disruption and replacement of follicles, as a part of perifollicular hyaline thrombosis and hemorrhage. The latter occurred in 19 mice dying on the sixth to eighth days, and follicular hemorrhage accompanied it in 13 of them. In 9 mice there was more or less profuse perifollicular proliferation of large cells with leptochromatic nuclei, and in 3 of these a small amount of nuclear debris was scattered in and among these cells. This "reticuloendotheliosis" may be of the same granulomatous nature as the proliferative reactions replacing necrotic areas in the liver.

The splenic pulp was moderately congested in most animals and contained variable amounts of myeloid tissue, in which more or less numerous erythroblasts, normoblasts and megakaryocytes were usually identifiable. Myelocytes and metamyelocytes were less often found. The myelosis in general seemed less in mice killed on the third and fourth days than in those examined at later periods. In 23 mice there were focal to patchy hyaline thrombosis and karyorrhectic necrosis of the pulp. Again this was confined to animals dying on the sixth to eighth days. It accompanied the perifollicular hemorrhage, thrombosis and organization in 12 mice. No special association with route of inoculation was descernible with either of these lesions.

In the Humphreys virus series, follicular hyperplasia, karyorrhexis and phagocytosis were less prominent, and the follicular status scarcely varied from that of the controls. Again the pulp was moderately congested and contained a considerable amount of myelopoietic tissue. Focal perifollicular hemorrhagic and hyaline thrombosis was observed in 2 mice; focal hyaline and karyorrhectic thrombosis of the pulp, in 5 of the 47 mice. These focal lesions occurred in mice killed on the sixth to twelfth days.

Rivers and Scott [4] found no noteworthy splenic lesions in mice, while Traub [5] noted enlargement up to as much as sixfold, with follicular hyperplasia and infiltration of the pulp by lymphocytes and mononuclear cells. Traub's reports of lymphocytic infiltration of the pulp and megakaryocytosis in virus-carrying recovered mice suggest resumption of normal erythromyelopoiesis. Traub's guinea pigs showed no lesions. Ours [3] showed some polymorphonuclear leukocytosis in the pulp from the fourth to the eleventh day, pulpal reticuloendotheliosis after the first week and erythrophagia and hemosiderosis in the second and third weeks. Intrafollicular phagocytosis was also present. Similar but somewhat less pronounced changes in guinea pigs were recorded by Perrin and Steinhaus.[9] In our monkeys [2] inconstant follicular phagocytosis and early congestion and lymphoid cell infiltration of the pulp were the only changes. Findlay and Stern [6a] reported some enlargement of follicles and congestion of pulp in mice, and some swelling of reticuloendothelial cells and of follicles in monkeys.

Bone Marrow.—The sections represented 75 mice and were from the tibia, the femur or both in 63 instances, the vertebrae in 14, the ribs and the pubis in 1 each. Generally the marrow was solidly cellular, sometimes congested, more often not. Mature leukocytes were generally more numerous in mice killed on the third day than in those killed later, myelocytes and ring nucleus metamyelocytes were generally the most numerous cells, and erythropoiesis appeared more prominent from the fifth day on than earlier.

Focal lesions (one focus of karyorrhectic necrosis and four small hemorrhages) were noted in 5 mice dead on the seventh and eighth days.

With the Humphreys strain, the vertebral marrow was again cellular, polymorphonuclear leukocytes were numerous in about half of the infected and the control mice, and myeloblasts were perhaps as frequent. Slight patchy phagocytosis of nuclear debris was noted in 2 infected mice, foci of hemorrhagic coagulation necrosis

in 2 more, foci of hemorrhage in another and vague patches of rarefaction in a sixth. No focal lesions of marrow were seen in the 24 controls, compared with 6 of 46 infected mice.

Our monkeys [2] showed no significant changes; our guinea pigs,[3] relative polynucleosis in the first and second weeks. Perrin and Steinhaus [9] noted irregular cellular pyknosis, karyorrhexis and depletion in guinea pigs the first week. Neither in mice nor in monkeys did Findlay and Stern [6a] observe significant changes in marrow.

Lymph Nodes.—Lymph nodes from 87 mice were studied. They were usually found accidentally, attached to other viscera. A single node had no identifying tissue with it. Included were mesenteric and omental nodes from 15 mice, retroperitoneal, perirenal and pelvic nodes from 17, mediastinal nodes from 50 and cervical nodes from 61.

In general, two series of changes were seen, the one follicular, the other involving pulp and sinuses. Follicular changes comprised swelling and hyperplasia, dilatation of lymph clefts and accumulation of nuclear fragments in these and in the swollen and phagocytic littoral cells. These changes appeared in the cervical lymph nodes as early as the third day after intracerebral inoculation, a day later after intraperitoneal. In mediastinal and abdominal nodes they were infrequent before the sixth day with either route of infection. In the 5 mice surviving ten to twenty days they were slight or absent.

The lesions of pulp and sinuses comprised serum and fibrin exudation in focal areas, often subcapsular, accompanied by more or less cellular depletion, hemorrhage, karyorrhexis, erythrophagia and frank coagulation necrosis. These changes occurred only in animals dead on the fifth to eighth days. Sometimes they coexisted with follicular changes; in some mice they may have obscured follicular reactions by the extent of the necrosis, but more often nodes showed either the one or the other process. In some mice cervical nodes showed this process on the fifth day, and in almost all, on the sixth and seventh days, after intracerebral inoculation. After intraperitoneal inoculation this type of reaction was less pronounced and did not appear until the seventh day.

Conversely, similar changes were more frequent and appeared earlier (sixth day) in mediastinal nodes after intraperitoneal inoculation and were less pronounced and less frequent after intracerebral. The relatively few abdominal nodes studied showed little difference attributable to route of inoculation.

Sinuparenchymal and follicular lesions showed fairly similar frequencies in the various groups of nodes studied. Uninvolved nodes were most often encountered in the cervical and mediastinal groups because of the routine sectioning of submaxillary gland and mediastinum.

Edema, fibrin exudation, necrosis, focal hemorrhage and, most often, diffuse and perivascular lymphocyte and, perhaps, plasma cell infiltration of periglandular tissues were observed about most of the more severely involved nodes. They appeared earlier and more often about cervical nodes after intracerebral inoculation and about mediastinal nodes after intraperitoneal inoculation.

In the Humphreys strain series, nodes were found in 41 mice: 33 cervical, 29 mediastinal, 25 mesenteric and abdominal and 6 perirenal.

Intrafollicular karyorrhexis and phagocytosis occurred chiefly between the fourth and the eighteenth day and were more frequent in cervical and abdominal than in thoracic nodes. No difference was assignable to difference in route of inoculation.

Focal areas of serous to serofibrinous exudate with more or less cellular depletion were seen in the pulp on the sixth to tenth days. This lesion was seen in cervical nodes from 6 mice, in thoracic from 4 and in abdominal from 3. It did not occur in the nodes from 23 control mice.

Periglandular edema and lymphocytic infiltration were seen in 2 mice; lymphocytic infiltration alone, in 2 more.

Follicular karyorrhexis and phagocytosis occurred with usually less marked intensity and about half the frequency in the control mice, and more often in those inoculated intracerebrally than in those inoculated intraperitoneally.

Traub [5a] noted in mice small lymph nodes with inconstant reticuloendothelial hyperplasia after intravenous inoculation only. In other mice and in guinea pigs he saw no lesions. In monkeys we [2] noted follicular and sinus endothelial hyperplasia with phagocytosis of neuclear debris, occasionally erythrocytes and more often hemosiderin. Follicular hyperplasia with phagocytosis of nuclear debris was noted in our guinea pigs [3] and in those of Perrin and Steinhaus.[9] Sinus reticuloendotheliosis, erythrophagia and hemosiderosis were less frequent in both series.

Adrenal Glands.—The sections represented 66 mice. Congestion of the reticular zone was noted in 11 mice killed on the third to sixth days; focal hemorrhage was present in 1 of these. Phagocytes laden with acid-fast, basophilic, yellowish brown pigment were seen in the reticular zone in 4 mice. In 2 mice dead on the seventh day coagulation necrosis of cells of the cortical parenchyma was seen. One had scattered, isolated cells involved in this process; the other, much of the reticular zone. In the latter animal there was associated lymphocytic infiltration. Three other mice, all surviving nine days or more, presented lymphocytic infiltration of the cortex and 1 of them infiltration of the medulla as well. Another of these 3 mice showed epithelioid cell granulomas with the lymphocytic infiltration.

In 1 of the aforementioned mice with interstitial lymphocytic infiltration and in 2 others there was infiltration of the periglandular fat by lymphocytes, and in another the surrounding fat presented diffuse edema, karyorrhexis and infiltration by lymphocytes and macrophages.

Necrosis was not seen in mice infected with the Humphreys strain, but diffuse and focal lymphocytic infiltration appeared in 19 of the 33 adrenal glands, involving the medulla in 14, the zona reticularis in 10, the zona fasciculata in 1, the zona glomerulosa in 7 and the periglandular tissues in 2. This infiltration was absent in 17 controls.

Findlay and Stern [6a] noted the occasional presence of a few lymphocytes in the adrenal glands of mice.

In their rats and guinea pigs lymphocytic infiltration was constantly present. Similarly lymphocytic infiltration was present in half of our guinea pigs,[3] while Perrin and Steinhaus [9] reported this lesion present in less grade in only 6 animals, and Traub [5a] observed no adrenal lesions. Focal hemorrhage appeared in 1 of our guinea pigs.

As to monkeys, we [2] reported focal lymphocytic infiltration in 4, hemorrhage in 2 and focal cortical necrosis in 1 of 22 animals, while Findlay and Stern [6a] described lymphocytic infiltration, denser in the cortex, and necrosis of individual cortical cells.

Kidneys.—Sections were made of kidneys from 108 mice, 13 dead on the third day, 12 on the fourth, 9 on the fifth, 25 on the sixth, 23 on the seventh, 6 on the eighth, 5 on the ninth and tenth and 4 on the twelfth to twenty-eighth days; 11 were from the 1934 series, whose survival time records were lost.

Usually there occurred more or less swelling, cloudiness and granular degeneration of the epithelium of the convoluted tubules, particularly of the proximal group, and in 40 of 76 kidneys on which fat stains were made, there was more or less pronounced deposition of fine fat droplets in the base of the epithelium, again more often in proximal than in distal convoluted tubules. Traces of fatty change were found in 4 mice on the third day. On the fourth to sixth day, fatty changes were seen in 12 of 20 mice inoculated intraperitoneally and in 5 of 20 inoculated intracerebrally. On the seventh to ninth days nearly all mice, however inoculated, showed more or less fatty changes. In 1 killed at twelve days and 1 at twenty there was no fat stained.

In many of the mice killed on the sixth and seventh days, glomerular cells seemed somewhat swollen, and the endothelium of arterioles at the bases of glomeruli was unduly prominent.

Interstitial and periarterial infiltration of the renal cortex by lymphocytes and sometimes plasma cells as well appeared in 30 mice. Occasional foci were seen in 2 mice on the third day, more in 4 on the sixth day, in 11 of 23 dead on the seventh day, in 3 of 11 on the eighth to tenth days. The 5 mice surviving eleven to twenty-eight days showed slight to dense irregular interstitial and denser periarterial infiltration. Similar lymphocytic infiltration was seen also in the pelvic mucosal and fatty tissues, and again was most pronounced in the 3 mice surviving twenty, twenty and twenty-eight days, respectively.

These infiltrations appeared in the 1934, 1939, 1943 and 1945 series.

In 1 mouse, dead eight days after intracerebral inoculation, there were red corpuscles, hemoglobin globules and hemoglobin casts in the tubules.

Klossiella muris infection was noted in 3 of the 108 mice, 1 in 1934, 2 in 1939.

In the Humphreys strain material only the kidneys of the 28 mice killed from the first to the fourteenth day were stained for fat. Moderate to fairly severe deposition of fine fat droplets in the epithelium of both proximal and distal convoluted tubules was noted in 5 of the 6 intraperitoneally inoculated mice killed on the sixth to tenth days. After intracerebral inoculation, the fatty degeneration was less intense, being designated with such terms as "traces" or "slight" in regard to 5 of 8 animals examined on the sixth to twelfth days. Correspondingly, the epithelium of convoluted tubules was more or less swollen and finely granular in the first two weeks, regardless of the route of inoculation.

Swelling of vascular endothelia was noted in mice killed on the sixth to tenth days, and there were a few necrotic cellular thrombi in 1 of these. On the sixth and eighth days lymphocytic infiltration of the cortex appeared in half of the mice. This was denser about arteries. It was denser and present in all mice killed on the tenth to fourteenth days. Periarterial concentration of the infiltration was more pronounced from the eighteenth to the forty-fourth day. After intracerebral inoculation the periarterial infiltration appeared earlier, and after intraperitoneal injection the diffuse interstitial infiltration cleared up to a greater extent in the eighteen to forty-four day period. In the ten to fourteen day period both the periarterial and the diffuse interstitial infiltration were approximately equal for the two routes of infection.

The focal and the diffuse lymphocytic infiltration of the pelvic mucosa were of variable density and appeared earlier and persisted longer in the intracerebrally inoculated series, though they were about equal for the two routes of infection in the peak ten to fourteen day period. In about half of the mice of both series there was focal lymphocytic infiltration of the pelvic fatty tissues from the sixth day on.

Traub [5a] reported lymphocytic infiltration of the kidneys of mice only after intravenous inoculation, and Findlay and Stern [6a] only occasionally observed a few lymphocytes. In virus-bearing mice killed ninety to two hundred and twenty-five days after infection, Traub [5b] observed interstitial nephritis, which, from his pictures, was comparable to the more severe infiltrations in our series.

In rats and guinea pigs Findlay and Stern noted lymphocytic infiltration as constantly present. Two thirds of our guinea pigs [3] presented interstitial and perivascular lymphocytic infiltration of the renal cortex, and many showed pelvic infiltration as well. More or less pronounced degenerative changes were present in convoluted tubules in our series; in the series studied by Perrin and Steinhaus [9] these were the only lesions, and in Traub's [5a] guinea pigs no lesions were observed.

As to monkeys, cortical infiltration appeared in two thirds of our animals,[2] and in some pelvic infiltration was present. The infiltrating cells were chiefly lymphocytes, but among them were plasma cells and fewer monocytes. Similar but less pronounced changes were noted by Findlay, Alcock and Stern [6b] in their series, and Findlay and Stern [6a] recorded glomerular endothelial swelling and the presence of infiltrating lymphocytes and plasma cells.

In 1 case of lymphocytic choriomeningitis in man Smadel, Green, Paltauf and Gonzales [11] reported pelvic hemorrhage.

Bladder.—In 2 male mice there was focal lymphocytic infiltration of the overlying serosa; in 1, the serosa was densely infiltrated by lymphocytes, and the surface epithelial cells of the mucosa were swollen and their cytoplasm was foamy and vacuolated. Four males and 8 females showed no lesions. In 7 females there was focal lymphocytic infiltration of the mucosa, the muscularis or both. Focal hemorrhage of the mucosa was present in 1 of these; seropurulent exudate was seen on the epithelial surface, with leukocytes emigrating through the epithelium, in another, and active mucosal lymphoid follicles were noted in 2 others.

The most pronounced reactions occurred in 1 mouse dead on the third day, in 2 on the sixth, in 3 on the seventh, and in 1 killed after twenty-eight days, while no lesions were present in 14 mice which died on the third to eleventh days.

In the Humphreys virus series, 15 of 20 mice killed on the eighth to the forty-fourth days presented more or less pronounced lymphocytic infiltration, of the mucosa in 14, the muscularis in 5 and the serosa in 4. Insterstitial serous exudate appeared in the mucosa in 2 mice and focal hemorrhage in 1 of these 2. No lesions were seen in the 6 control mice.

Among our guinea pigs,[3] focal lymphocytic infiltration of the mucosa appeared in 5 and in some was accompanied by fibroblastic or endothelial proliferation or focal hemorrhage. Perrin and Steinhaus [9] noted similar focal lesions in 3 guinea pigs, and in 2 others they observed, respectively, focal vascular thrombosis and mucosal edema.

Three of our monkeys [2] presented cystitis characterized by focal hemorrhage, concentric vascular endothelial proliferation and swelling, and perivascular and diffuse lymphocytic infiltration. Similar mucosal lymphocytic infiltration alone was seen in 2 others, and mucosal edema and serosal granulomas in 1 each. The remaining 6 of the 13 monkeys presented no lesions of the bladder.

Male Genitalia.—Sections of testes from 48 mice were studied, including the epididymides of 40. In 1 mouse there was moderate lymphocytic and plasma cell infiltration of both the testis and the epididymis and in a second a relatively slight lymphocytic infiltration of both organs. In 10 other mice there was focal lymphocytic infiltration of the epididymis alone, more often subcapsular than insterstitial, and in 1 of these and in 4 others focal lymphocytic infiltration was present in the polar fat or the spermatic cord. In 1 mouse there was dense infiltration of the lower pole of the epididymis by polymorphonuclear leukocytes, monocytes and lymphocytes, with epithelial necrosis and desquamation. The last was considered as an intercurrent process, being found three days after intracerebral inoculation.

The testes generally presented active spermatogenesis and those of 46 mice were recorded as normal. The epididymis was normal in 27.

In 3 mice, 2 with epididymo-orchitis, 1 with epididymitis, there was also irregular interstitial lymphocytic infiltration of the prostate, paraurethral glands and seminal vesicles. The prostate was normal in 3 mice; the seminal vesicle, in 5.

In the Humphreys virus series, lymphocytic infiltration of the epididymis appeared in variable grade in 16 of 37 mice, while all 44 testes were normal. In 9 mice there was irregular lymphocytic infiltration of the polar fat, and in 9 the cremaster muscle, the spermatic cord or both presented serosal, perivascular or interstitial infiltration by lymphocytes. A single focus of lymphocytic infiltration of the polar fat was the only lesion of testicle or cord in 21 control mice.

The prostates from 4 mice and the seminal vesicles from 2 of these presented slight to moderate diffuse and perivascular lymphocytic infiltration. A single control showed no lesions.

In our guinea pigs [3] there were single instances of focal perivascular lymphocytic infiltration and endothelial proliferation in the cremaster muscle and the epididymis and occasional similar lesions in the seminal vesicles. The testes presented moderate degenerative changes. Perrin and Steinhaus [9] observed no testicular lesions in their series.

Moderate diffuse and perivascular infiltrations by lymphocytes and fewer plasma cells appeared in 9 of 15 monkeys in our series. The epididymis was most often involved, then the cremaster muscle, the spermatic cord and the testis.

Female Genitalia.—In 2 mice the ovaries showed irregular interstitial and serosal lymphocytic infiltration. Both of these mice also presented lymphocytic infiltration of the endometrium. Five ovaries were recorded as normal, while 3 showed focal karyorrhexis of thecal epithelium or of lutein tissue, and 2 showed focal accumulation of basophilic acid-fast pigment in stromal phagocytes.

The uterine tubes of 8 mice were recorded as normal, while in those of 4 there were areas of lymphocytic infiltration of the serosa and the muscularis,[4] of the muscularis and of the muscosa.

The uteri of 5 mice were normal, and in the uteri of 6 there was moderate to dense lymphocytic infiltration of the serosa, the muscularis or the endometrium, most often of the serosa and the muscularis together. The uterus of 1 contained a cystic papillary tumor.

In 6 mice the vagina was normal; in 2 there was focal lymphocytic infiltration of mucosa or muscle.

All but 3 showed more or less lymphocytic infiltration of pelvic subperitoneal tissues.

The Humphreys virus series included only 2 female mice. One, killed one day after intraperitoneal inoculation, showed no lesions of the vagina, the cervix, the uterus or the uterine tube. In the other, killed on the eighth day, there was perivascular to diffuse lymphocytic infiltration of the broad ligament and the tubal serosa. The muscularis and the mucosa of the tube were not involved.

Perrin and Steinhaus [9] studied the female genitalia in 24 guinea pigs and observed no significant lesions. With respect to monkeys,[2] we reported focal lymphocytic infiltrations as constantly present, involving the tubal mucosa most often, then the uterine and tubal musculature, the parametrium and the ovary.

SUMMARY

Visceral reactions, infrequent after intracerebral inoculation, become much more prominent when other routes of inoculation are employed.

The more striking gross and histologic observations in mice are these: Polyserositis with serous exudate involves the pleura and the peritoneum. Fatty degeneration of the liver and, to a less extent, of the kidneys appears as early as the third day and persists into the third week. Focal necrosis occurs in the liver and, rarely, the adrenal cortex and the corpora lutea. More diffuse and irregular but sometimes focal necrosis is seen in the thymus, the spleen, the lymph nodes and the bone marrow. Scattered capillary thrombi occur in the liver and other viscera. Generalized lymphocytic infiltrations, sometimes including larger lymphoid and plasma cells, some macrophages and fewer polymorphonuclear leukocytes, involve serosal tissues of the pleura and the peritoneum, the renal cortex and pelvis, the liver, the salivary glands, the pancreas, the lungs, the heart, the adrenal glands and less frequently the tissues of the rest of the gastrointestinal and genitourinary tracts, as well as the originally reported locations of the meninges, the choroid plexus, the ependyma and, in addition, the spinal ganglions.

There is considerable variability in the tissues and organs involved from mouse to mouse, often with no apparent reason. While severe visceral reactions appear earlier and more often with noncerebral inoculation, they occur also with cerebral inoculation. Meningoplexal reactions appear later with noncerebral inoculation but in individual instances may be quite as severe as those with cerebral inoculation. It is noteworthy that involvement of the cervical lymph nodes is earlier and more frequent after intracerebral inoculation.

In general the mice inoculated with the Humphreys virus showed less cellular necrosis, edema and fibrin and more lymphocytic infiltration.

Otherwise the lesions correspond in detail, organ by organ, with those obtained with other old and recent strains of the virus of lymphocytic choriomeningitis.

From the fact that similar visceral lesions appear not only in mice but also in intracerebrally inoculated monkeys and guinea pigs, it appears that the visceral lesions are a general effect of the virus, not peculiar to any one species. Since in mice such visceral reactions appear earlier and more often after other than cerebral inoculation, and since natural routes of infection are generally other than neural, it appears that spontaneous infections should produce visceral lesions proportionately more often than cerebral inoculation does and that such lesions might well dominate the pathologic picture in natural infection.

It has been observed that persons who have had no illness suggesting a septic or lymphocytic meningitis may possess protective antibodies in their blood serums against this virus. Further, Armstrong and Hornibrook [14] reported that the virus of lymphocytic choriomeningitis was recovered from the blood of a patient with an influenza-like syndrome, on the fifth day of illness, and that the serum contained protective antibodies after, but not before, the illness in question.

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EXPERIMENTAL ENDOCARDITIS (RHEUMATIC-LIKE AND BACTERIAL) IN RATS

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Typical anatomic rheumatic endocarditis has not yet been observed to occur spontaneously in any animal as it is seen in man, but endocarditis produced experimentally in rats has a marked similarity to the lesions in the human heart valves. The white rat was therefore selected for the experiments reported in this paper.

The purpose of the experiments is to explain, if possible, the relationships between human rheumatic endocarditis and bacterial endocarditis and to obtain further information relative to the genesis of the two types of endocarditis.

RELATIONSHIPS

Acute rheumatic endocarditis and bacterial endocarditis are closely related both clinically and anatomically.

Clinically, the acute rheumatic endocarditis commonly passes gradually into the bacterial type. This observation has been made frequently by clinicians, especially in patients who were in the latter part of the second and in the early part of the third decade of life. A second clinical observation is that in at least 90 per cent of the cases of bacterial endocarditis there is a background of a rheumatic valvular lesion either acute or partially or completely healed (Christian [1]; Gelfman [2]). A third observation is that bacterial endocarditis is the primary cause of death in about 30 per cent of all the cases of rheumatic valvular deformities (Gelfman [2]; Clawson [3]).

Anatomic relationships between the two types of endocarditis have repeatedly been observed. Both lesions are present on the same valve in from 80 to 100 per cent of the cases examined (Clawson [4]; Von Glahn and Pappenheimer [5]). The two lesions are similar except in size and in infection of the platelet thrombus in the bacterial type. The infected thrombus is the distinguishing characteristic of bacterial endocarditis.

Two interpretations have been advanced to explain these clinical and anatomic relationships: (1) The two types are different diseases involving the same valve at the same time, the bacterial type being (*a*) a secondary infection on a healed or nearly healed valve deformity or (*b*) a secondary infection of an acute rheumatic vegetation (Von Glahn and Pappenheimer [5]), and (2) they are the same disease etiologically, with different clinical and pathologic manifestations (Clawson [4]).

Which of these two interpretations is correct cannot be answered definitely in human cases.

GENESIS

The chief problem in the genesis of acute rheumatic or bacterial endocarditis is *what enters into the valve to bring about the characteristic reaction in each type.* The following substances or products of immunologic processes have been suggested or implied:

1. Proteins of bacteria, as Streptococcus viridans or Streptococcus haemolyticus with or without allergy. There is nothing anatomically characteristic of an inflammation associated with bacterial allergy except the degree (Maximow [6]; Clawson [7]). A small amount of infection in an allergic animal will produce as great a degree of inflammation as a larger amount of infection in a nonallergic animal.

2. Toxins, such as those of Str. haemolyticus (Coburn [8]).

3. A toxic or active product liberated in an antigen-antibody reaction; i. e., (*a*) the agglutination of organisms, (*b*) the action of precipitins, with liberation of a toxic substance which causes a local reaction especially about blood vessels (Opie [9]), and (*c*) allergic reactions (hypersensitiveness). There are two types of hyper-

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sensitiveness which have been applied in the study of the genesis of acute rheumatic endocarditis, the bacterial or delayed type and the anaphylactic or immediate type. The bacterial form of allergy in rheumatic fever has been studied by Swift,[10] Birkhaug,[11] Gibson, Thomson and Stewart[12] and others. It has been shown by intradermal tests that a high percentage of patients with acute rheumatic fever are allergic to streptococci or streptococcic products and that patients with bacterial endocarditis are not allergic. This suggests that patients having bacterial endocarditis had previously been allergic but had been desensitized by the blood stream infection.

A point to be considered in thinking of the role of delayed hypersensitiveness in rheumatic endocarditis is whether the rheumatic child was allergic before contracting rheumatic fever, or whether allergy exists as a result of the rheumatic infection or of intercurrent infections with increasing age. Birkhaug's[13] observations showed that 20 per cent of normal children and 72 per cent of rheumatic children were allergic to streptococci. Gibson, Thomson and Stewart,[12] by skin tests with extracts of both Str. haemolyticus and Str. viridans, found that the percentage of allergy rose from the age of 5 years to that of 15 years, at which it ceased to increase in both rheumatic and control children. At 15 years of age there was practically no difference in the frequency and the degree of allergy to Str. haemolyticus extract (68 per cent rheumatic and 66 per cent controls), but there was a greater difference with the Str. viridans extract (46 per cent rheumatic and 19 per cent controls). They concluded that their work did not add much support to the allergic theory of acute rheumatic fever. The same conclusion was reached by Jones.[14] Clawson and Wetherby[15] found that 50 per cent of normal medical students give a positive reaction to Str. viridans in cutaneous tests.

The anaphylactic or immediate type of allergy fits in with the assumption that a streptococcic toxin or other soluble proteins cause an anaphylactic reaction in from two to three weeks after the initial inoculation and bring about a reaction in the valve similar to that seen in the Arthus phenomenon. Coburn,[8] following a series of studies on the relation of Str. haemolyticus to the causation of acute rheumatic fever, concluded that a substance is released, presumably from the antibody-producing tissues, which directly or indirectly alters mesodermal structures. He also stated that this substance is probably not an infectious organism and that at the present time there is no evidence to suggest that it is viable. This anaphylactic mechanism in the genesis of acute rheumatic heart disease apparently is accepted by many at the present time.

The lesions closely simulating Aschoff nodules which were observed by Rich[16] in the hearts of rabbits following injections of horse serum are explained by him on an anaphylactic basis.

THE PROBLEM

Since the relationships of acute rheumatic and bacterial endocarditis and the possible substances or products of immune processes which cause the inflammation in the valves in the two types of endocarditis cannot be determined definitely in human cases, the question arises whether it is possible to obtain additional information from experiments on animals. Can either or both types of valvulitis be produced by forced methods from a single inoculation or a short course of inoculations with a specific substance in an animal in which endocarditis seldom or never occurs spontaneously? An attempt is made in the following experiments to answer this question and also to study some of the foregoing considerations concerning the relationships and the genesis of the two types of endocarditis.

Incidence of Acute Rheumatic-like Endocarditis and Bacterial Endocarditis in Rats Inoculated with Various Substances and by Various Methods

Substances Injected	Number of Rats	Acute Rheumatic-like Endocarditis		Bacterial Endocarditis		Both	
Str. viridans (intracardiac)	94	38	40.4%	5	5.3%	5	5.3%
Str. haemolyticus (intracardiac)	75	23	30.6%	11	14.6%	2	2.6%
Str. viridans (intraperitoneal)	10	0	0	0	0	0	0
Str. haemolyticus (intraperitoneal)	30	0	0	0	0	0	0
Str. viridans antigen (intracardiac)*	10	0	0	0	0	0	0
Dick toxin (intracardiac)	10	0	0	0	0	0	0
Rabbit serum (intracardiac)	10	0	0	0	0	0	0
Horse serum (intracardiac)	10	0	0	0	0	0	0
Egg white (intraperitoneal)	5	0	0	0	0	0	0
Str. haemolyticus (subcutaneous, in agar)	26	0	0	0	0	0	0
Control animals	46	0	0	0	0	0	0

* Undenatured streptococcus antigen (Lilly) containing 5 mg. of nitrogen per hundred cubic centimeters.

EXPERIMENTS

A summary of the experiments is seen in the accompanying table.

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16. Rich, A. R., and Gregory, J. E.: Bull. Johns Hopkins Hosp. **73**:239, 1943.

One strain of Str. viridans was used. It was isolated from the blood of a patient suffering from an attack of acute rheumatic fever. There were three strains of Str. haemolyticus. Two had been typed as Lancefield A types. The third was not typed. It had recently been isolated from an abscess. The Str. viridans antigen was prepared by Eli Lilly and Company from the same strain of Str. viridans which was used in the experiments. The Dick toxin was from stock toxin on the market for immunizing purposes.. The white rat was the animal employed in all of the experiments.

Both Str. viridans and Str. haemolyticus were injected into the ventricular cavity, generally the left (0.5 cc. of a broth culture, from one to six times, on an average three times, at weekly intervals). The Str. viridans antigen, the Dick toxin and the rabbit and horse serums were also injected into the ventricular cavity, but the time between the injections was two weeks. Three injections of 0.5 cc. each were given except with the Dick toxin, of which there were only two, of 13,500 skin test doses each. Two cubic centimeters of egg albumin (10 per cent) was injected into the peritoneal cavity three times at intervals of two weeks, and the animals were killed a week after the last injection. Str. viridans and Str. haemolyticus were also injected intraperitoneally (1 cc. at each injection) from five to fifteen times at intervals of two to twelve weeks. Str. haemolyticus heavily seeded in agar at 45 C. was injected subcutaneously in 2 cc. quantities twice at intervals of two weeks. The purpose of the intraperitoneal and subcutaneous injections was to see if toxins or products of the organisms, especially of the hemolytic strains, might directly or by allergy produce an injury in the valve.

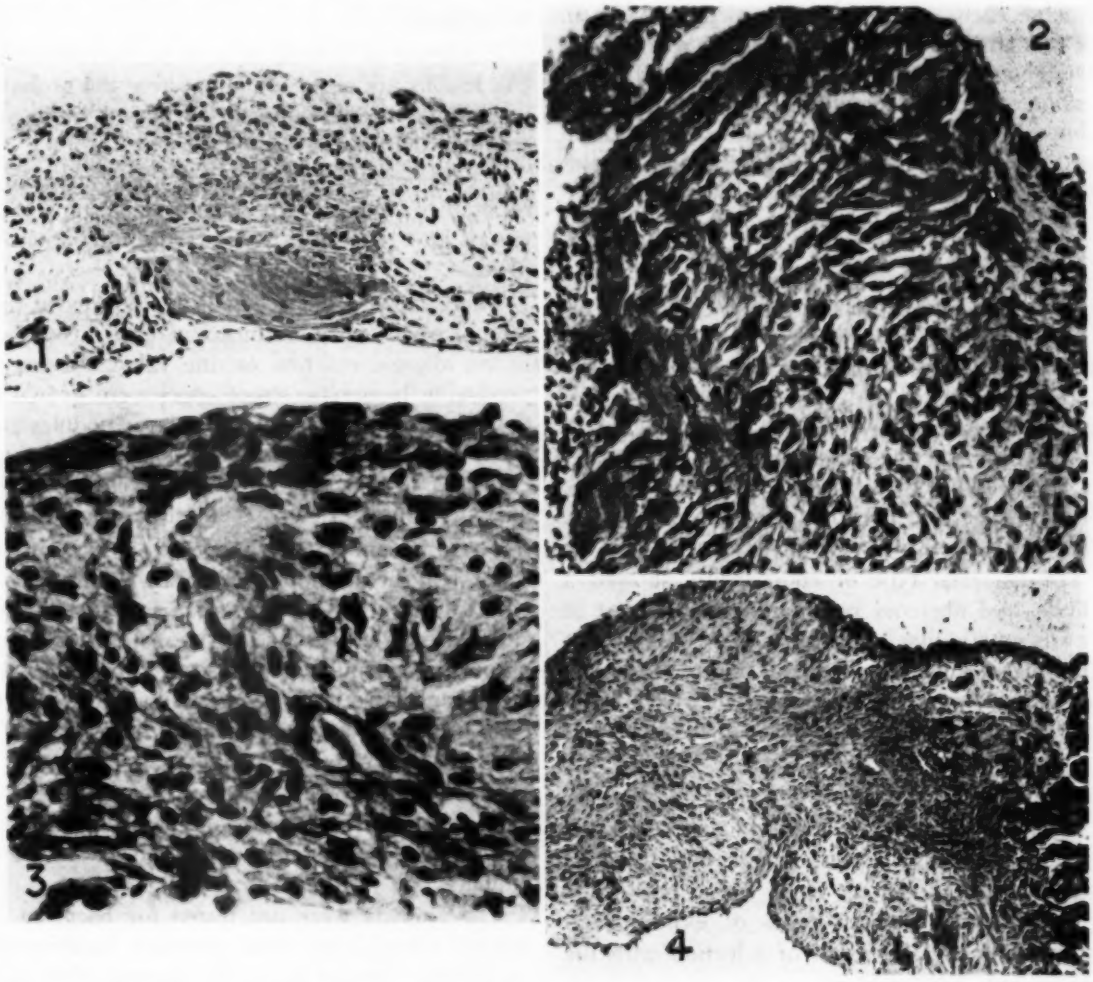

Fig. 1.—Early rheumatic-like valvulitis showing fibrinoid material. Str. haemolyticus was injected.

Fig. 2.—Rheumatic-like endocarditis with fibrinoid material and proliferative inflammation. Str. viridans was injected.

Fig. 3.—Section of midvalve region with cellular reaction and some fibrinoid reaction. Str. viridans was injected.

Fig. 4.—A mitral valve. Note the fibrinoid reaction at the base. There is healing with early scar formation in the valve. Str. viridans was injected.

RESULTS

In the table it is noted that endocarditis, acute rheumatic-like or bacterial endocardial lesions, developed only when the whole organisms were put into the blood stream. The animals which had been inoculated so as to produce an anaphy-

lactic shock, as with serums, egg albumin or Dick toxin, had no valvular injuries. The animals given intraperitoneal injections and those given subcutaneous injections of streptococci in agar, which was to hold the organisms locally and permit the toxin to diffuse into the blood stream, likewise had no valvular injuries.

The rheumatic-like lesions found on the valves (the mitral, the aortic and the tricuspid in order of frequency) had a definite resemblance to the verrucous lesions on the human heart valves in acute rheumatic fever. Microscopically, as in the human lesions, the inflammation was present throughout each involved valve (fig. 3). The hyaline fibrinoid material, emphasized by Klinge [17] and others as characteristic, was within the valve (figs. 1 and 2). The cellular inflammatory reaction occurring with the fibrinoid change was chiefly a proliferation (figs. 3 and 4). The cells were spindle shape or rounded usually, with abundant cytoplasm. They were generally mononuclear, but multinuclear forms were seen. As in human cases, the cells were apparently derived from fibrocytes, histiocytes, blood lymphocytes and a special class of histiocytes, the Anitschkow cells. When there was desquamation of the endothelium, a small platelet thrombus was formed. No bacteria were found in the valve or the thrombus. Definite healing was seen to occur in a few cases (fig. 4).

The bacterial type of endocarditis showed a cellular and fibrinoid reaction similar to that in the rheumatic-like vegetation, but the platelet thrombus, which was usually more extensive, was infected and had colonies of streptococci lying within the thrombus.

COMMENT

Some additional information concerning the relation between acute rheumatic and bacterial endocarditis is obvious from the experiments. It is evident that both types of experimental endocarditis are the result of infection with the same agent, Str. viridans or Str. haemolyticus. The possibility of the verrucous rheumatic-like vegetation being due to a previous cause can hardly appear likely.

Another fact to be noted is that bacteria are not found in the rheumatic-like lesions, the production of which was evidently started by the streptococci only a relatively short time before the lesions were examined. This should be considered when examining human rheumatic endocarditis.

The experimental lesions differ only in degree of injury and reaction in the cusps and in the larger vegetation containing colonies of streptococci in the typical bacterial endocarditis. The concept of Von Glahn and Pappenheimer [5] that the platelet thrombus in the rheumatic type becomes infected and produces the bacterial type appears to represent the probable course of events in these experimental lesions. The infection is evidently the same and the degrees of valvular involvement in the two types cannot be widely separated.

Evidences of healing in some of the rheumatic-like lesions are observed, suggesting the probable process in the development of healed rheumatic valvular deformities.

A considerable amount of additional knowledge is obtained from the experiments concerning what enters into the valves to start the inflammatory process.

There is no evidence favoring the theory that streptococcic toxin or antitoxin acts directly or by an allergic reaction on the valve, for toxin-producing hemolytic streptococci were grown in the animals in the subcutaneous agar nodules and in the peritoneal cavity, and the Dick streptococcic toxin was injected into the cavity of the heart without the development of valvular lesions.

There is some suggestion that agglutinins are a factor in the development of the valvulitis probably by agglutinating the organisms in the very small vessels in the valves. A greater percentage of positive results were observed following the second and third inoculations, and associated with these injections high agglutinating titers (1 : 6,400 to 1 : 25,000) were observed.

Precipitins probably were present in the animals receiving the toxins, serums and egg albumin, but no lesions were observed in these animals.

The animals were not tested for bacterial or delayed allergy, but from previous experiments on rabbits [7] it can be safely assumed that bacterial allergy was present only in the animals inoculated subcutaneously with streptococci in agar. The animals in which endocarditis developed in all probability did not have allergy of the bacterial type.

The anaphylactic type of hypersensitiveness resulting from inoculations with rabbit and horse serums, Dick toxin, streptococcic toxin and egg albumin did not stimulate any inflammatory change in the valves. In some of the animals, especially those given injections of the Dick toxin, definite and severe symptoms of anaphylaxis were observed.

17. Klinge, F.: Ergebn. d. allg. Path. u. path. Anat. **27**:1, 1933.

The influence of immune reactions on the genesis of endocarditis has not been interpreted conclusively. It is suggested that a high humoral antibody content favors the development of the valvular lesions by localizing the organisms in the valves and that the bacterial allergic state may favor the development of the bacterial type of endocarditis, for it has been found that with equal doses of streptococci a greater degree of tissue reaction occurs in animals having the delayed type of hypersensitiveness than in non-allergic animals. It may be possible that the absence of, or the low incidence or degree of, streptococcic hypersensitiveness in children and its high incidence in adults may be a prominent factor in the difference in the age incidence of acute rheumatic and bacterial endocarditis. Further observations and experiments are necessary before definite statements may be made.

The only substance in the experiments which obviously stimulated the inflammatory reaction in the valves was the bacterial protein itself, both that of Str. viridans and that of Str. haemolyticus.

CONCLUSIONS

Valvular lesions closely simulating acute rheumatic endocarditis anatomically can be produced in a high percentage of rats by injecting either Str. viridans or Str. haemolyticus into the blood stream.

Some of the rheumatic-like lesions show a degree of healing resembling the early healing observed in human rheumatic valvulitis.

Lesions similar to human bacterial endocarditis are produced on the same valve or on separate valves in association with the rheumatic-like vegetations, or the bacterial vegetations may occur independently.

These rheumatic-like and bacterial lesions are produced only when the bacterial organisms are in the blood stream. Endocarditis fails to develop following the injection of other proteins: Dick toxin, rabbit and horse serums, egg albumin and an extract of Str. viridans.

Agglutinins in the blood stream apparently favor the development of the valvulitis.

Hypersensitiveness (allergy), immediate (anaphylactic) or delayed (bacterial), does not appear to be a factor in the genesis of the endocarditis except possibly in the bacterial form of endocarditis, in which the delayed type of hypersensitiveness may have been an influencing factor.

These experiments support the theory that acute rheumatic endocarditis and bacterial endocarditis are etiologically similar but differ in degree of manifestation and that they occur as a response to a direct valvular infection with the bacterial cells.

STUDIES ON THE PATHOGENESIS OF GLOMERULONEPHRITIS

II. PRODUCTION OF GLOMERULONEPHRITIS IN RATS BY MEANS OF AUTOANTIBODIES TO KIDNEY

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SAN FRANCISCO

In a previous publication [1] evidence was presented that group A beta hemolytic streptococci are able in some way to render renal material antigenic in the same species. When mixtures of killed streptococci and homologous kidney emulsion were injected into rabbits and rats, antibodies developed in the serum which gave serologic reactions in vitro with plain normal homologous kidney.

The question arose whether these antibodies to kidney are able to produce renal lesions by means of their reaction in vivo with the kidney.

It is our purpose in the present paper to describe the methods which were used to demonstrate that these antibodies produce renal lesions and to present the results of various experiments which throw some light on the mechanism which is operating in this connection.

The clinical and pathologic aspects of the renal lesions produced are described in part III of these studies, which immediately follows this part.

MATERIALS AND METHODS

Tissues.—Kidney and other tissues used were perfused until they were free of blood as described in the previous publication.[1] The material was ground either in a mortar with sand or in a Waring Blender. All tissue emulsions were made up to a concentration corresponding to 20 Gm. of fresh tissue in 100 cc. of 0.85 per cent saline solution. The content of dry solids of the suspension was about 20 mg. per cubic centimeter. The tissue emulsions were stored, frozen solidly, at a temperature of —76 C. in the carbon dioxide ice box.

Streptococci.—The streptococci were grown for about forty-eight hours on a synthetic protein-free medium described by Bernheimer,[2] the acids formed being neutralized periodically. A heavy growth was obtained. The organisms were collected by centrifugation and resuspended either in a smaller volume of the culture fluid or in isotonic solution of sodium chloride. The suspension was adjusted to a concentration of 2 per cent packed organisms when centrifuged, which corresponded to approximately 3 mg. of dried bacteria per cubic centimeter. These streptococcic suspensions also were kept frozen in the carbon dioxide ice box.

Serologic Method.—The collodion agglutination technic used in these experiments for the detection of antibodies to kidney has been described in detail in previous publications.[3]

Clinical and Pathologic Methods.—The evaluation of the results of the experiments was carried out predominantly on the results of the urinalysis and the histologic observations. The urinalysis consisted of a quantitative determination of the urinary protein and the Addis count. The details of these methods are described in part III.

Materials Used in Immunization.—In the main, the materials used for immunization were prepared as follows:

1. Group A streptococci, strain N. Y. 5, were killed by adding 10 per cent of volume of ether and keeping the mixture in the refrigerator for twenty-four hours. Tests for sterility were set up. This suspension of killed streptococci (containing approximately 3 mg. of dried bacteria per cubic centimeter) was admixed to an equal volume of 20 per cent emulsion of rat kidney.

2. Several other strains of group A streptococci (type not determined) were similarly treated and admixed to emulsion of rat kidney.

3. Organisms of the strains mentioned in the two preceding paragraphs, living, were admixed to emulsion of kidney in the quantities already indicated. The mixture was then precipitated with three times its volume of acetone at a temperature of about —60 to —70 C. and subsequently washed with acetone and dried. Before injection the powder was resuspended by grinding it in a mortar and adding the original volume of saline solution.[4]

4. Similar antigenic mixtures were precipitated and dried in the same manner with alcohol instead of acetone.[4]

5. Similar mixtures were precipitated with alum at room temperature, washed and resuspended in saline solution.[4]

6. Living organisms were mixed with emulsion of rat kidney.

7. Streptococci were grown on rat kidney for twelve to forty-eight hours and then were killed by the addition of ether.

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1. Cavelti, P. A., and Cavelti, E. S.: Arch. Path. **39**:148, 1945.

2. Bernheimer, A. W.; Gillman, W.; Hottle, G. A., and Pappenheimer, A. M., Jr.: J. Bact. **43**:495, 1942.

3. Cavelti, P. A.: J. Immunol. **49**:365, 1944. Cavelti and Cavelti.[1]

4. The organisms were found to have been killed during procedures 3, 4 and 5.

As controls, the following materials were used: (1) an emulsion of rat kidney alone, (2) streptococci alone, killed and living, and (3) killed streptococci mixed with an emulsion of rat heart in amounts similar to those of the organisms admixed with emulsion of kidney.

The total number of rats treated with antigens consisting of streptococci mixed with kidney was approximately 250. For the control experiments about 120 rats were used.

Schedules of Immunization and Dosage.—Almost all injections were intraperitoneal. Generally it was found necessary to discontinue the administration of the antigen after a relatively short period because prolonged immunizations such as were carried out in the earlier studies, in which injections were made twice weekly for a few weeks to several months, appeared to be inadequate for the production of renal lesions, although this method yielded higher titers of antibodies to kidney in the blood as determined by serologic reactions in vitro.

A grouping of the animals with respect to the main schedules of immunization and the materials employed follows:

Group A: Each rat received a single injection of streptococcus-kidney mixture. The doses ranged from 0.5 cc. to 10 cc.

Group B: A single schedule was used, consisting of four to ten injections of streptococcus-kidney mixture given on successive days. The total amount given varied between 0.5 cc. and 20 cc. In the majority of cases, however, the total amount was between 1 and 5 cc.

Group C: In this group the single injection of streptococcus-kidney mixture or the series of four to ten injections on successive days was repeated after an interval of one month or more. This group also contained animals which received several such repeated treatments after similar intervals. The total doses were similar to those in group B.

Group D: The rats received single and repeated injections of rat kidney alone. The doses varied up to 10 cc.

Group E: The rats received single and repeated injections of streptococci alone, killed or living. The doses varied up to 10 cc.

Group F: The rats received single and repeated injections of streptococci admixed to emulsion of rat heart. The doses varied up to 10 cc.

TIME OF ONSET AND TYPES OF URINARY EVIDENCE OF RENAL INJURY FOLLOWING IMMUNIZATION OF RATS

In all cases there was a significant interval between the immunization and the onset of the pathologic urinary excretion. When a single injection was given, this interval was at least eight to ten days. When a schedule of four to ten injections given on successive days was employed, the interval in the great majority of the cases was two to three weeks, counted from the first injection. Thus, the urine remained normal for at least a few days after the conclusion of the schedule of immunization, usually for about one to two weeks, and in 1 case even for five weeks. The urinary findings are described in part III, but they should be mentioned here briefly. It appeared that in the main two types of changes could be distinguished.

Type 1 (glomerulonephritis).—The findings consisted of proteinuria, cylindruria (various types of casts), hematuria and the presence of large numbers of renal tubular epithelial cells and some leukocytes in the urine. In the urinary samples from many rats these findings slowly decreased and disappeared within a period of about two to eight weeks. In those of another proportion of the animals, however, they persisted for the whole life of the rat, and the proteinuria increased heavily in the later stages.

Rats presenting these urinary abnormalities showed histologically definite glomerular lesions, especially when the proteinuria was marked.

Type 2 (no glomerular lesions but possibly some tubular damage).—In this group proteinuria was absent or insignificant. The casts, although occasionally present in the urine in great numbers, were almost exclusively of the cellular variety. Renal tubular cells were also present, and hematuria, if observed at all, was usually of a low degree. The changes had a tendency to disappear in a relatively short time. They never led to chronic progressive lesions.

In rats presenting these abnormalities of the urine, definite glomerular lesions were not found, but tubular changes were occasionally seen.

It should be emphasized that the interval between immunization and the onset of urinary changes previously described applies to both of these types. In the following pages there will be reference to these two types of urinary changes.

EFFECTS OF VARIOUS PREPARATIONS OF ANTIGEN

Significant urinary abnormalities were obtained with the highest degree of consistency either when streptococcus-kidney mixtures (organisms killed with ether) were injected as such or when mixtures of streptococci and kidney were injected which had been precipitated with acetone, dried and the material resuspended.

The streptococcic strain N.Y.5 was used most extensively. Some other strains of group A streptococci appeared to have about the same activity in producing renal lesions by means of antibodies to kidney, whereas a number of others seemed to be weaker in this respect.

Preparations consisting of streptococci grown on kidney and subsequently killed led only rarely to renal lesions, although definite lesions in a few rats were obtained by this method.

Living organisms mixed with kidney also gave poor results with respect to the production of

renal lesions. It must be pointed out, however, that only much smaller amounts of living organisms could be injected, as larger amounts seemed to induce septic processes in the animals.

Effects of Various Doses of Streptococcus-Kidney Mixture (Killed Organisms) Given in Single and Repeated Injections.—When a single injection was given, relatively large doses, such as 5 or 10 cc., had to be employed to obtain urinary changes. The incidence of changes was relatively low, and in most instances they were of type 2. In a few instances urinary changes of type 1 (glomerular lesions) were obtained after a single injection. In 1 case persistent and apparently low grade progressive changes were noted over many months.

Much better results were obtained with the schedules of immunization in which a dose was injected daily for four to ten consecutive days.

The incidence of intense urinary changes (both types) with such a mode of treatment increased from about 30 per cent of the animals when the daily dose was small (a total of 0.5 cc. for the whole schedule of immunization) to 100 per cent when it was large (a total of 10 cc. to 20 cc.).

The intensity of the lesions in terms of quantitative urinary findings failed to show consistent parallelism with the amounts of the antigen used; this was especially true with respect to lesions of type 1. On the whole, however, larger doses led more frequently to severe renal lesions.

In many animals in which the urinary changes had subsided or had been greatly reduced or in which urinary abnormalities had failed to appear after the first treatment, the same immunization with streptococcus-kidney mixtures (either a single injection or injections of four to ten divided doses on consecutive days) was repeated after at least one month but more often after several months. Many of the animals then again produced pathologic urine. The changes again developed after an interval of one to two weeks, although on the whole this interval appeared to be somewhat shorter than after the first treatment. There was a greater tendency toward the development of changes of type 1 and of progressive lesions. In any group of animals treated at the same time, the highest incidence of acute lesions of type 1 (definite glomerulonephritis) was 50 per cent, and the highest incidence of subacute or chronic progressive lesions was 30 per cent. There were also animals which, after recovery from the renal lesions resulting from the first schedule of immunization, consistently failed to present urinary changes again on repetition of the immunization.

A number of rats which already had slight or moderately severe persisting nephritis received additional immunization with the streptococcus-kidney antigen several months after the onset. Some of these showed exacerbation of their urinary symptoms. The exacerbation in some appeared to be transitory in that the urinary findings soon went back to their original level. In others, however, marked aggravation and acceleration of the disease seemed to result. There also were rats in which no exacerbation of the nephritis occurred.

In some rats with repeated injections of large amounts of streptococcus-kidney mixtures, development of aseptic peritonitis with formation of abscesses and extensive peritoneal adhesions was observed. There was no correlation between this peritonitis and the development of nephritis. On the contrary, the majority of such rats failed to present nephritis, although they sometimes showed urinary changes of type 2.

RESULTS OF THE CONTROL EXPERIMENTS

Injections of rat kidney alone, even when large amounts were used and many repetitions of the injections made, failed to produce any urinary changes whatsoever.

Injections of streptococci alone, when moderate doses were used, also failed to yield urinary abnormalities although these doses were much larger than the minimal doses necessary to produce changes when the organisms were given in combination with kidney. Repetition of the injections of moderate doses after an interval of about a week also failed to lead to urinary changes.

The results were somewhat different with repeated injections of large doses of streptococci alone. Such treatments were followed in some of the animals by urinary changes of type 2, which also appeared after an interval of one to two weeks. The changes were of lower intensity and of shorter duration than the type 2 changes obtained with streptococcus-kidney mixtures, and they occurred in a lower incidence. Furthermore, urinary changes of type 1 (glomerulonephritis) have never been obtained by means of injections of streptococci alone, not to speak of progressive lesions.

The animals given injections of mixtures of streptococci and rat heart behaved similarly to the ones treated with streptococci alone with respect to urinary changes. Some changes of type 2 were obtained with large doses of the antigen, but again neither acute nor progressive glomerular lesions were seen.

RESULTS OF SEROLOGIC STUDIES

A large part of the animals used in the experiments described were bled after various intervals. In several groups, bleedings were carried out every two to three days during the first two weeks after immunization and subsequently every five to seven days for a period up to two months. Blood (0.5 to 1 cc.) was withdrawn by syringe from the tail vein at each bleeding after the rat had been warmed. The serums were tested for antibodies to rat kidney by means of the collodion agglutination test according to the technic described elsewhere.[3] As already mentioned, high serologic reactivity was consistently obtained only when the immunizations were carried out over long periods during which two weekly injections were given. However, antibodies to kidney also were frequently demonstrated in the experiments described here. Of 135 rats (excluding the group given alum-precipitated antigen as mentioned in the next paragraph) whose serums were tested repeatedly, 74 gave samples which produced no reactions or doubtful ones with rat kidney in vitro, and 61 gave samples one or another of which, often several, produced a definite reaction. The titers usually ranged between dilutions of serum of 1 : 20 to 1 : 160, but sometimes higher titers, up to 1 : 1,280, were noted. The interval between immunization and appearance of antibodies to kidney as demonstrated in vitro showed wide variations, namely, from about four days to three or four weeks. Of the 61 rats with positive serologic reactions, 44 showed renal lesions, either transitory or progressive. The onset usually occurred at or shortly after the time during which antibodies to kidney were observed in the blood. Some rats, in which subsequently severe subacute or chronic nephritis developed, gave serum which showed relatively high titers of antibodies to kidney and particularly intense agglutination of the sensitized collodion particles. However there were also some rats with renal lesions whose serum showed no definite reaction with rat kidney.

In one group of 30 rats, which had been given streptococcus-kidney antigen precipitated with alum in a schedule of six injections on consecutive days, high titers of antibodies to kidney (dilution of serum 1 : 160 to 1 : 1,280) were recorded for most of the animals. The peak of antibodies in this group appeared rather late, twenty-five days after the first injection. In only 1 of these rats did a significant renal lesion develop.

From the rest of the animals immunized with streptococcus-kidney antigen, blood was not obtained for serologic examination. This group includes many of the rats in which severe renal lesions developed.

Rats treated with rat kidney alone never showed any evidence of antibodies to kidney in the blood.

Rats treated with streptococci alone also generally failed to produce antibodies to kidney. In a few, which had been treated repeatedly with large doses or given living streptococci, some antibodies to kidney appeared to have been formed. Some of these were the same rats in which slight urinary changes also developed.

Rats treated with mixtures of streptococci and rat heart did not show antibodies to kidney in the blood.

COMMENT

In a previous paper [1] evidence was presented that injections of mixtures of streptococci and homologous kidney lead to formation of antibodies to the kidney.

In this paper it is reported that such procedures when carried out under proper conditions also lead to renal lesions.

It appears, therefore, that these antibodies to kidney can act as a pathogenic agent and produce renal lesions by means of their reaction with the kidney in vivo. The evidence obtained so far that the lesions produced were actually due to pathogenic action of the antibodies to kidney and not to sensitization of the kidney to streptococcic products and subsequent allergic reaction or to primary toxicity of the streptococcic products can be summarized as follows:

1. There is always an interval of time of considerable length between the immunization and the appearance of the urinary symptoms. This interval averages about seven to fourteen days and appears to correspond to the time necessary for the production of antibodies to kidney. It would be difficult to conceive of a primary toxic action of streptococcic products manifesting itself as late as two weeks after the administration of the toxin.

2. There was some, although by no means complete, correlation between the presence of antibodies to kidney as demonstrated in vitro and the development of nephritis. When the fact is considered that the kidneys receive about one third of the total blood supply of the body, it can be expected that these antibodies to kidney are rapidly absorbed in the kidney and therefore generally do not accumulate in the blood to give high titers. As demonstrated by Sarre and Wirtz,[5] nephrotoxin, i. e., antibodies

5. Sarre, H., and Wirtz, H.: Klin. Wchnschr. **18**: 1548, 1939.

to rabbit kidney prepared in the duck, when injected into the rabbit is bound by the kidneys in a few minutes. Nephritis failed to develop in one kidney the artery of which had been clamped during and for ten minutes after the intravenous injection.

3. Treatment with streptococci alone or with these organisms in combination with tissues other than kidney does not lead to significant renal lesions.

4. Renal lesions have been obtained by means of a single injection of streptococcus-kidney mixture.

5. There is definite lack of the parallelism between the dosage of the antigen and the development of renal lesions that would be expected if the lesions were due to toxic effects of the antigen. Furthermore, in animals which had been treated previously with the antigen, immunity to the toxins could be expected to develop and thereafter there would be less tendency for such lesions to be produced by reinjection. This was not found to be the case.

As will be discussed further in part III, which immediately follows this part, the urinary changes referred to here as type 1 represent glomerulonephritis. The significance of the changes of type 2 is not well understood. The fact that they appear so late after immunization seems to speak against their toxic origin, and the fact that they are most easily obtained by means of streptococcus-kidney mixtures would appear to favor the view that they are due to some sort of antibodies to kidney. It is conceivable that several different antibodies to kidney are formed on treatment with streptococcus-kidney mixtures, some of the inciting antigens coming from the glomeruli and others perhaps from the tubules and other structures in the kidney. Thus the lesions might differ according to the predominance of one or the other type of antibody to kidney.

As already pointed out by us in part I of this series [1] and earlier by Schwentker and Comploier,[6] human glomerulonephritis might be conceived as due to autoantibodies to kidney. Such a mechanism would imply the formation of a kidney-streptococcus antigen, perhaps as a result of toxic streptococcic products acting on the kidney during the height of the streptococcic infection which precedes human glomerulonephritis. This streptococcus-kidney antigen then would initiate the formation of autoantibodies to kidney, and these antibodies by their reaction with the kidney would precipitate glomerulonephritis. Such a conception would furnish a perfect explanation of the peculiar interval of time of about two to three weeks between streptococcic infection and onset of nephritis in man. The formation of antibodies to kidney and the development of nephritis due to these antibodies has been shown to occur in animals when they are treated with streptococcus-kidney antigen. It remains to be demonstrated that streptococci can effect the formation of streptococcus-kidney antigen in vivo and, furthermore, with respect to human nephritis, that such a mechanism actually is operating.

SUMMARY

Immunization of rats with mixtures of killed group A streptococci and emulsion of rat kidney leads to formation of antibodies to rat kidney.

These antibodies to kidney act as a pathogenic agent and produce glomerulonephritis by means of their reaction with the kidney in vivo.

6. Schwentker, F. F., and Comploier, F. C.: J. Exper. Med. **70**:223, 1939.

STUDIES ON THE PATHOGENESIS OF GLOMERULONEPHRITIS

III. CLINICAL AND PATHOLOGIC ASPECTS OF THE EXPERIMENTAL GLOMERULONEPHRITIS PRODUCED IN RATS BY MEANS OF AUTOANTIBODIES TO KIDNEY

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In the preceding paper (part II of these studies) the production of experimental glomerulonephritis in rats by means of autoantibodies to kidney was described. The essentials of the procedure consisted in immunization of the animals with mixtures of killed group A streptococci and perfused kidney of the same species. As a result of the ability of the streptococcus to render renal material antigenic in the same species, antibodies to kidney are formed which can be demonstrated by in vitro reactions. These autoantibodies to kidney, by their reaction with the animal's own renal substance, lead to the development of glomerulonephritis. In this paper the clinical features ofthis experimental nephritis are described together with the pathologic aspects.

MATERIALS AND METHODS

Strain of Rats Used.—Practically all the rats used for these experiments were originally derived from the Evans strain and had been bred in our laboratory. Albino rats, black and white hooded and brown and white hooded rats, as well as brown, gray and black rats, were employed.

Diet and Environment.—About 60 per cent of the diet consisted of Purina Dog Chow; the remaining 40 per cent was composed of scratch, oats, bread and fresh carrots. Water was supplied freely. The room available for the keeping of the rats had a fairly constant temperature of 28 C. (82.4 F.).

Collection of Urine.—The examination of urine was based on Dr. Thomas Addis' quantitative procedure for the determination of protein, and the Addis sediment count (modified for rats). To collect timed urine specimens, each rat was placed in an individual cage, the bottom of which consisted of a funnel made of galvanized iron covered with a fine wire mesh. The outlet of the funnel had a diameter of about 3 to 4 mm. and led into a suitable glass container which was closely covered by the funnel. Evaporation of the urine thus was reduced to a minimum. Two drops of a 6 per cent solution of formaldehyde was put into the glass containers. The rats were kept in these cages overnight for twelve hours. No food was given during this period, to avoid contamination of the urine, but about 10 cc. of water was supplied each in a small suspended bottle. As the urine under these conditions usually was slightly alkaline, some of the casts and perhaps red blood cells might have been destroyed. However, when the urine was not too concentrated, such elements usually were still present when the sediment was examined. In the later experiments a solution containing 0.3 per cent of ammonium chloride and 0.1 per cent of sodium chloride was given instead of drinking water for thirty-six hours before and during the collection of urine. The majority of the specimens then were slightly acid, and the casts and the red blood cells were well preserved. The ammonium chloride was not given to rats that were seriously ill with nephritis. Its administration was also avoided several days prior to the determination of the blood urea levels.

Urinalysis.—The volume of each twelve hour specimen of urine was noted. All urinary findings were recorded in terms of the quantity excreted in twenty-four hours, which varied from about 3 to 15 cc. The specimens were centrifuged for three minutes at 3,500 revolutions per minute, and the supernatant of each was drawn off until 0.5 cc. was left. The supernatant fluid was used for the determination of protein by Addis' method, for which special tubes were used.[1] Into each tube 1 cc. of centrifuged urine was placed; to this was added 3 cc. of distilled water and 2.5 cc. of Tsuchiya's reagent (1.5 Gm. of phosphotungstic acid, 5 cc. of concentrated hydrochloric acid and 95 per cent ethyl alcohol to make a total of 100 cc.). The contents of the tubes were well mixed and centrifuged for five minutes at 3,500 revolutions per minute. The volume of the sediment in each was noted, and the result was read as milligrams of protein per cubic centimeter of urine from Addis' table, corrections for temperature being made. The final value was expressed as milligrams of protein excreted in twenty-four hours.

When the twelve hour specimen of urine was too small to allow a quantitative determination of the protein, a qualitative estimation was made with sulfosalicylic acid.

Sediment Count.—The sediment of the centrifuged urine was resuspended in the 0.5 cc. of supernatant left in the tube, by means of a capillary pipet and a sample was placed in a Neubauer counting chamber. The types of casts were noted and the number of casts, the number of red blood cells and the number of leuko-

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From the George Williams Hooper Foundation for Medical Research and the Division of Medicine of the University of California Medical School.

1. MacKay-McNaught-Sheffly-Stafford tubes were used, which have been described by J. P. Peters and D. D. Van Slyke (Quantitative Clinical Chemistry: II. Methods, Baltimore, Williams & Wilkins Company, 1932, pp. 683-684).

cytes and renal tubular epithelial cells together were recorded in terms of the numbers excreted in twenty-four hours.

Urinary Findings in Normal Rats.—Urinalyses were carried out for approximately 500 normal rats; this number included the animals which were to be used for the experiments on nephritis. For a considerable number of rats three urinalyses were made at intervals of at least four days. Later on, one, and in all doubtful cases several, such preexaminations were made. However, as the urinary results usually remained unchanged during and for some time after the immunization, several normal analyses were recorded for most of the animals.

Although the findings in the urine of the individual rat were fairly consistent, some variation was found among individual rats and particularly among different strains of rats. In a group of rats originally derived from the Wistar strain, microscopic hematuria was frequently noted, and many of the animals also showed slight proteinuria and some casts. But the rats which were used almost exclusively in the present experiments and which were derived from the Evans strain and bred in our laboratory gave much better results. In the majority of them the protein excreted in twenty-four hours amounted to about 1 to 3 mg., and no elements were found in the sediment, with the exception of a few leukocytes in some cases. The upper limits of normal were set somewhat arbitrarily as follows: protein, not more than 6 to 7 mg. per twenty-four hours; casts, not over 6,000 per twenty-four hours; red blood cells, not over 200,000, and leukocytes and renal tubular epithelial cells together, not over 250,000 per twenty-four hours. Approximately 80 per cent of our rats had urinalyses corresponding to these standards; the others were rejected

URINARY FINDINGS IN RATS WITH EXPERIMENTAL NEPHRITIS

As already pointed out in the preceding paper, the urine remained normal for an average period of about one to two weeks after the immunization with streptococcus-kidney antigen.

Acute Phase.—The urinary symptoms in the acute phase of the renal lesions consisted of proteinuria, cylindruria, hematuria (mostly only microscopic) and the presence of renal tubular cells and leukocytes in the urine. The quantities of the pathologic constituents varied considerably. The most consistent findings in the early stage were casts and renal tubular cells. The number of casts varied from about 20,000 to 4,000,000 per twenty-four hours. Various types of casts were present. The majority were usually cellular and granular casts. When the proteinuria was marked, large numbers of hyaline casts were also present. Casts consisting mainly of red blood cells were but rarely found. The number of renal tubular epithelial cells, including leukocytes, ranged from about 400,000 to 20,000,000 per twenty-four hours. The leukocytes made up only a small fraction of this number. The number of red blood cells excreted per twenty-four hours varied greatly, between 400,000 and about 70,000,000. Gross hematuria corresponded to values of more than 10,000,000 red cells per twenty-four hours. Some rats with definite nephritis had almost total absence of red blood cells in the urine. Sometimes marked hematuria was noted for one or a few days at the very onset of the disease. The proteinuria in the initial stage varied between 10 mg. and about 60 mg. per twenty-four hours. Whereas the sedimentary findings reached their peak soon after the onset, the proteinuria often showed a slower rise.

The majority of the rats with nephritis tended to recover. The proteinuria and the sedimentary findings slowly decreased and disappeared within a period of from several weeks to about two months. The sedimentary findings often persisted for a longer period than the proteinuria.

As stated in the preceding paper, two types of urinary findings were distinguished. The findings just described correspond to type 1. The changes designated as type 2 were characterized particularly by lack of proteinuria. The sedimentary findings of this type consisted mainly of cellular casts and renal tubular cells. Microscopic hematuria was usually absent or insignificant. The changes were quantitatively not pronounced in most cases, and they disappeared soon, within a few days to about two weeks after their onset. The changes appear to correspond to mere tubular damage and are perhaps not very specific.

Subacute and Chronic Phase.—In a number of rats the pathologic urinary findings persisted throughout life and showed certain characteristic alterations. The proteinuria increased more or less rapidly over a period of months. It reached its peak always as late as several months after the onset. Values up to 500 mg. per twenty-four hours have thus far been recorded. All rats with persistent proteinuria also showed abnormal sedimentary findings, although these were quantitatively less than in the acute phase of the disease. The casts were predominantly of the hyaline and granular type. Microscopic hematuria, sometimes only intermittent, was present in most cases.

Latent Phase.—After the acute phase had passed, the only finding which persisted in many rats was slight proteinuria, sometimes not associated with any abnormal sedimentary findings and, as such, of doubtful significance. In other rats this slight proteinuria was accompanied by constant or intermittent microscopic hematuria and presence of a few casts. After weeks or, more often, many months there developed in a few of these rats the urinary findings described for the subacute and chronic

TABLE 1.—*Examples of Urinary Findings Considered Typical*

Time After Onset	Protein Mg. per 24 Hr.	Casts per 24 Hr.	Red Blood Cells per 24 Hr.	Leukocytes Plus Tubular Cells per 24 Hr.
Acute Phase Followed by Healing				
Shortly before onset.....	1.5	0	0	0
At onset.....	6.0	320,000	150,000	500,000
After onset:				
2 days.....	10.4	400,000	300,000	1,000,000
4 days.....	22.4	1,170,000	2,000,000	2,000,000
10 days.....	13.4	110,000	750,000	350,000
21 days.....	7.0	224,000	350,000	300,000
1 mo.....	2.7	46,000	800,000	100,000
2 mo.....	2.0	20,000	150,000	100,000
3 mo.....	3.0	0	200,000	100,000
4 mo.....	1.3	0	0	0
Acute Phase Ending in Death				
Shortly before onset.....	3.0	0	0	0
At onset.....	20.2	720,000	500,000	1,250,000
Acute Phase Becoming Latent				
Shortly before onset.....	4.0	0	100,000	0
At onset.....	18.1	160,000	700,000	750,000
After onset:				
2 days.....	23.8	1,600,000	1,000,000	4,800,000
4 days.....	33.0	800,000	1,000,000	8,000,000
7 days.....	38.4	1,900,000	1,300,000	4,000,000
10 days.....	31.0	800,000	850,000	3,200,000
14 days.....	14.2	4,000,000	9,200,000	20,000,000
21 days.....	7.3	900,000	4,800,000	3,400,000
1 mo.....	4.3	80,000	800,000	700,000
2 mo.....	20.0	10,000	2,600,000	250,000
3 mo.....	4.3	8,000	300,000	200,000
4 mo.....	14.2	13,000	150,000	200,000
5 mo.....	11.5	10,000	200,000	100,000
6 mo.....	15.5	7,800	300,000	100,000
Acute Phase Becoming Subacute but Terminating in Death				
Shortly before onset.....	1.9	0	0	0
At onset.....	++	140,000	500,000	400,000
After onset:				
2 days.....	++	33,000	250,000	100,000
7 days.....	11.3	53,000	600,000	200,000
10 days.....	54.7	50,000	750,000	300,000
14 days.....	21.6	40,000	300,000	100,000
1 mo.....	15.2	20,000	250,000	150,000
2 mo.....	87.8	40,000	500,000	300,000
3 mo.....	156.0	33,000	300,000	300,000
Acute Phase Becoming Chronic and Terminating in Death				
Shortly before onset.....	4.4	4,000	120,000	200,000
At onset.....	30.2	80,000	660,000	100,000
After onset:				
2 days.....	16.4	1,120,000	300,000	1,200,000
4 days.....	28.6	320,000	600,000	400,000
10 days.....	28.1	100,000	200,000	300,000
14 days.....	40.9	160,000	240,000	300,000
1 mo.....	37.5	400,000	1,000,000	800,000
2 mo.....	99.6	120,000	500,000	300,000
3 mo.....	237.9	170,000	500,000	150,000
4 mo.....	119.4	350,000	200,000	1,400,000
5 mo.....	50.4	466,000	500,000	2,400,000
Acute Phase Becoming Chronic and Terminating in Death				
Shortly before onset.....	2.6	0	120,000	0
At onset.....	19.2	33,000	4,400,000	100,000
After onset:				
7 days.....	26.7	22,000	4,000,000	300,000
14 days.....	21.3	40,000	660,000	530,000
1 mo.....	146.7	60,000	3,800,000	600,000
2 mo.....	72.0	70,000	2,000,000	200,000
3 mo.....	148.8	52,000	1,300,000	2,000,000
4 mo.....	471.6	370,000	1,000,000	1,900,000
5 mo.....	227.4	93,000	500,000	400,000
Acute Phase Becoming Latent and Then Chronic				
Shortly before onset.....	2.4	0	0	0
At onset.....	19.4	18,000	200,000	100,000
After onset:				
4 days.....	16.6	80,000	1,500,000	60,000
10 days.....	13.7	3,000	400,000	50,000
21 days.....	9.0	20,000	300,000	100,000
1 mo.....	12.6	6,000	1,000,000	50,000
2 mo.....	13.2	6,000	200,000	0
3 mo.....	6.2	0	0	0
4 mo.....	76.4	26,000	300,000	300,000
5 mo.....	288.0	20,000	300,000	200,000
6 mo.....	378.0	30,000	300,000	300,000

phase, i. e., severe proteinuria along with more or less pronounced sedimentary findings. These findings developed even in some animals which seemed to have recovered completely from the acute phase in that for a considerable period they had failed to present urinary abnormalities.

In a considerable number of other rats, constant or intermittent slight proteinuria and sedimentary findings persisted after the acute phase, without aggravation, or with final disappearance after months. (The urinary findings of some cases considered typical are given in table 1.)

ADDITIONAL CLINICAL FINDINGS

At the onset of the acute phase there was often pronounced oliguria, which sometimes lasted for many days. Definite edema, particularly around the eyes, and general puffiness were observed in some cases at the same time. Rats with severe acute urinary findings often appeared sick and lost much weight.

In the rats with severe chronic proteinuria, ascites and edema were frequently noted. There sometimes was definite pitting edema of the legs and tail. In some of these rats hypoproteinemia was observed, with serum protein values as low as 2.7 per cent, as determined by a gravimetric method employing solutions of copper sulfate. Lipemia was also noted in a few of these rats, extremely marked in 1.

In many rats which died or were killed when they appeared to be seriously ill, the blood urea was found to be elevated, being between about 80 mg. and 250 mg. per hundred cubic centimeters of serum. High blood urea levels were noted in both the acute and the subacute or chronic phase of the disease.

In the subacute or chronic phase, anemia was usually marked. The lowest hematocrit value observed was 36.6 per cent of normal.

Extreme emaciation developed in many of the rats with subacute and chronic renal disease.

PATHOLOGIC OBSERVATIONS IN THE ACUTE PHASE

Gross Examination.—The kidneys were usually markedly pale except in the very early stage, when they appeared red or dark and congested. Often they were swollen and edematous. Their weight was often considerably above normal, as indicated by Addis' charts on normal organ weights, showing an increase to from 130 to 184 per cent of normal. The heart weights were significantly elevated in about one half of the rats showing the acute phase, lying between 110 and 147 per cent of normal, indicating the presence of hypertension. (The weights of the

hearts and the kidneys computed as percentages of normal are given in table 2.)

Histologic Examination.—(*a*) Glomeruli: All the glomeruli were affected. Under low power magnification, they were obviously somewhat enlarged, and all or most of them had an increase in number of nuclei. Under high power magnification it was seen that this was due chiefly to an increase in the number of endothelial and epithelial cells of the tuft. Such proliferation was seen already in early stages, a few days after the onset of the urinary changes. In many cases the individual cells of the tuft appeared greatly swollen. Owing to the swelling and the increase in the number of epithelial cells, the space between the capillary loops and lobules was often filled by them. Similarly, the endothelial cells protruded into the lumens of the capillaries, and some of them seemed to have desquamated into the lumen. As a result of the proliferation of the endothelial cells, capillaries sometimes were seen to be lined with several layers of these cells. Besides desquamated endothelial cells, the lumens of the glomerular capillaries often contained polymorphonuclear leukocytes and mononuclear cells. In addition to these cellular contents, the capillary lumens were sometimes observed to contain debris or masses of homogenous appearance which when extensive obstructed or distended the loops. An intracapillary network of clumps and threads which stained blue with Mallory's technic was also noted. Resulting from the narrowing and obstruction of the lumens by the aforementioned process, there was usually present a more or less pronounced ischemia of the glomerular capillaries. The glomerular basement membrane, particularly later in the acute phase, appeared somewhat thickened. However, in the earlier stages most of the thickening of the

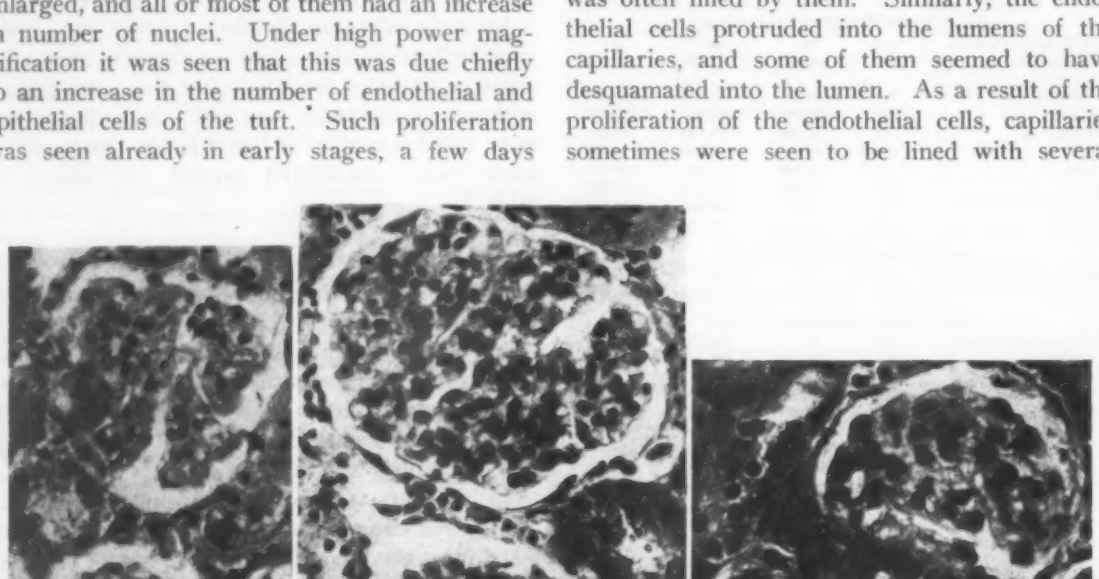

Fig. 1.—*A*, glomeruli of a normal rat kidney (× 370). *B* (rat 464), early acute stage. Duration two days. Glomeruli enlarged. Proliferation of the cells of the tuft as evidenced by the increase in the number of nuclei (× 370). *C* (rat 332), early acute stage. Duration three days. Marked swelling of the cells of the tuft. Glomerulus ischemic due to obstruction of the capillaries by protoplasmic masses. Exudate in Bowman's space. Slight proliferation of the cells of the tuft (× 500).

TABLE 2.—*Weights of Kidneys and Hearts*

	Rats with Experimental Nephritis					
	Controls*		Acute Stage		Subacute and Chronic Stage	
	Kidney	Heart	Kidney	Heart	Kidney	Heart
Number of rats for which weights were determined.....	25	25	22	20	15	15
Percentage of normal†						
Between 80 and 100	..	25	..	5	..	2
Between 100 and 110	6	..	2	3	..	1
Between 110 and 120	19	..	2	3	1	1
Between 120 and 130	..	..	6	2	1	6
Between 130 and 140	..	..	2	4	1	..
Between 140 and 150	..	..	..	3	2	1
Between 150 and 175	..	..	9	..	4	2
Between 175 and 200	..	..	1	..	5	..
Between 200 and 250	..	..	..	..	1	2
Weights significantly elevated (kidneys above 130%, hearts above 110%).....	..	..	12	12	13	12

* The controls include normal untreated rats, rats treated with streptococci only (killed and living) and rats treated with rat kidney only.

† Addis' charts of normal weights have been made the basis of the evaluations.

capillary wall seemed to be due to the swelling of the cells and their proliferation.

The epithelium of Bowman's capsule, besides swelling of the cells and occasional desquamation, often showed slight proliferative reactions, sometimes forming several layers of epithelium.

In Bowman's space there were often some desquamated epithelial cells, red blood cells and masses, clumps and threads of exudate. Between the tuft and Bowman's capsule in a large proportion of the glomeruli there were adhesions, which became more and more pronounced in the later stages of the disease.

(*b*) *Tubules and Interstitium:* In contrast to the changes in the glomeruli, the tubular changes were not widespread and mostly slight. The convoluted portions of the tubules were predominantly the site of the changes. Occasionally, areas of cloudy swelling, hyaline droplet degeneration or necrosis of the tubular epithelium were seen. Often, in the lumens of some of the tubules pathologic constituents were observed which consisted of cellular detritus, desquamated epithelial cells, red blood cells, leukocytes and hyaline and granular casts. In 1 case of the early acute phase the majority of the tubules were practically filled with casts.

The interstitium often contained infiltrates of inflammatory cells, mainly lymphocytes. These infiltrates were situated in the vicinity of the blood vessels, particularly the small arteries. Occasionally, periglomerular infiltrates of similar cells were present.

PATHOLOGIC OBSERVATIONS IN THE SUBACUTE AND CHRONIC PHASE

Gross Examination.—The kidneys were always pale. The color varied from pale gray to yellowish or mixed brown and gray. The surface was either smooth or, in a few cases, markedly granular. The enlargement of the kidneys was often grossly obvious. The renal weights ranged from slightly above normal to 222 per cent of normal. The majority of rats with subacute or chronic nephritis apparently had had hypertension, as evidenced by the cardiac weights, which were increased up to 238 per cent of normal.

The pleural and peritoneal cavities sometimes contained considerable amounts of clear pale or yellowish or slightly bloody fluid.

Histologic Examination.—(*a*) Glomeruli: On the whole, the changes were those of progressive obliteration of the glomeruli. Many of the glomeruli were considerably enlarged. Proliferation of the endothelial and epithelial cells of the tuft was evident. Numerous and widespread adhesions between the tuft and Bowman's capsule and also between the loops and the lobules of the tuft were present. According to the stage of the disease, various degrees and stages of hyalinization could be seen, more or less widespread, involving loops, lobules or whole glomeruli. Areas of beginning hyalinization still contained many nuclei, whereas later the nuclei disappeared more and more.

Ischemia of the glomeruli was regularly observed. The few remaining patent loops sometimes were congested with blood, but in many other cases even those loops which still contained lumens were almost devoid of red blood cells. The glomerular basement membrane always showed thickening, the degree of which appeared to parallel the stage of the process.

Proliferation of the capsular epithelium was in most cases not conspicuous; however, epithelial crescents, sometimes cellular, sometimes more or less hyalinized, were occasionally noted. At times, part of the crescent appeared to have been formed from material of the tuft.

The capsular space where not obliterated was usually free but sometimes contained desquamated epithelial cells, debris, red blood cells, fibrin or proteinic exudate.

(*b*) Tubules and Interstitium: The lumens of the tubules often contained some red blood cells, desquamated epithelial cells, leukocytes, detritus and casts of the hyaline and granular types. Casts were often seen in large numbers. The tubular epithelium frequently showed more or less widespread degenerative changes, such as cloudy swelling and hyaline droplet degeneration. Fatty degeneration of the tubules was revealed by fat stains; mostly it involved scattered groups of tubules; in 1 case it was more widespread.

Small areas of tubular atrophy were noted relatively early in the subacute phase. In cases in which it was thus observed there were occasional dilated tubules with flattened epithelium, mostly empty, but sometimes filled with large hyaline casts. In 2 cases in which the process was far advanced, large cystic tubules plugged with casts were present. Sometimes there were also areas containing hypertrophic tubules. In many cases of obviously severe glomerular lesions, the tubular changes described were not widespread. In other instances they were marked and in 2 cases severe.

The interstitial connective tissue showed various degrees of proliferation. As in the

case of the tubular changes, the proliferation in many rats was slight and present only in scattered areas; in others it was more pronounced and in 1 extremely marked. The interstitium was infiltrated more diffusely than in the acute stage, again mostly with lymphocytes. In addition, denser perivascular and occasionally periglomerular lymphocytic infiltrates were seen. (For a tabulation of some of the pathologic changes see table 3.)

small infiltrates were sometimes seen in the interstitium between the muscle fibers. In some cases of acute glomerulonephritis, extensive interstitial myocarditis was apparent, with numerous areas of destruction of the muscle fibers and infiltrates predominantly of leukocytes but also some lymphocytes. In 1 of these cases the liver showed numerous small hemorrhages. In some cases of advanced chronic nephritis, examination of the liver revealed extensive dif-

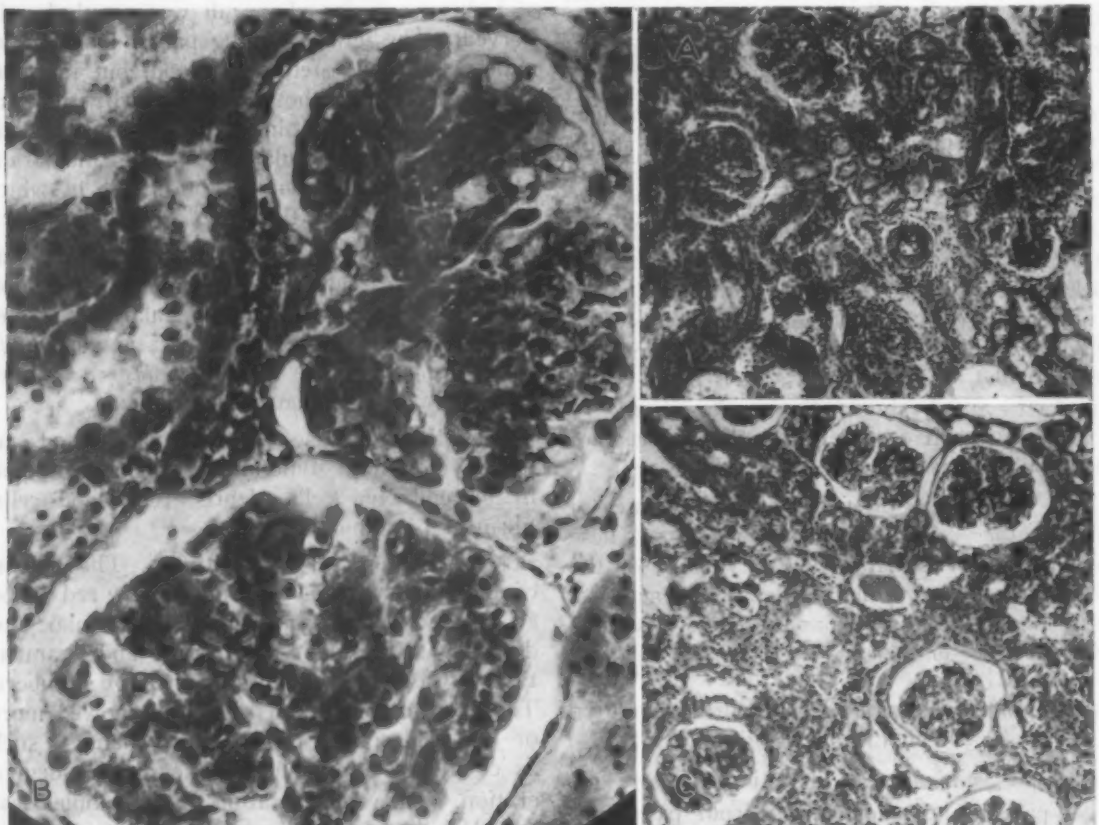

Fig. 2.—*A* (rat 39), late acute—early subacute stage. Duration sixty-eight days. Glomeruli enlarged. Marked proliferation of the cells of the glomerular tuft, obliteration of the glomerular capillaries, capsular adhesions. Slight increase of the interstitial connective tissue with slight interstitial round cell infiltration (× 75). *B*, same as *A* (× 370). *C* (rat 1), early chronic stage. Duration one hundred and fifty-seven days. Beginning hyalinization in glomeruli. Dilated tubules containing casts. Areas of tubular atrophy. Increase of the interstitial connective tissue, which is infiltrated with round cells (× 100).

HISTOLOGIC OBSERVATIONS IN OTHER ORGANS

Other organs examined in a proportion of the cases of nephritis were the heart, the lungs, the liver and the spleen. The changes appeared to be relatively insignificant. In many cases of both acute and chronic renal lesions perivascular infiltrates, consisting predominantly of lymphocytes, were found in all or several of these organs but particularly in the heart. Similar

fuse degenerative changes with small areas of necrosis. The lung sometimes contained interstitial infiltrates of leukocytes and lymphocytes. Some of the alveoli occasionally were filled with inflammatory cells. The spleen appeared normal except in a few cases in which small hemorrhages or small areas of necrosis were noted.

INCIDENCE OF THE RENAL LESIONS OBSERVED

A total of 250 rats were immunized with streptococcus-kidney antigen. In regard to 204

of these, sufficient clinical and pathologic data were available to allow their classification with respect to the renal lesions obtained (fig. 5).[2]

EXPERIMENTAL NEPHRITIS IN RABBIT

Despite the fact, reported previously,[3] that rabbits when immunized with mixtures of streptococci and rabbit kidney produced antibodies to kidney in high titers, nephritis failed to develop in that series of experimental animals with but few exceptions. Definite acute nephritis of a severe type developed in 1 rabbit. This rabbit had shown antibodies to kidney in a titer far exceeding all the others, namely 1:40,960. The onset of nephritis occurred one week after the last injection of the last series of injections for immunization. The clinical symptoms were proteinuria, microscopic hematuria and cylindruria. At the end of two weeks the animal died, after the determination of nonprotein nitrogen had shown 190 mg. per hundred cubic centimeters of serum. The histologic evidence of renal disease was marked, consisting in the main of proliferation of the cells of all the glomerular tufts, presence of leukocytes in the capillaries, exudation into the capsular spaces, extensive degenerative changes of the tubular epithelial cells, including necrosis, and marked broadening of the interstitium with edema and infiltration of inflammatory cells. In a few other rabbits, apparently similar but less intensive changes of the glomeruli were noted, but they did not seem to be sufficiently pronounced for one to ascertain their nature, particularly because the clinical evidence available was poor. The high alkalinity of the urine of rabbits renders the making of a reliable analysis extremely difficult.

In a considerable number of rabbits which had been given prolonged immunization, extensive chronic renal amyloidosis developed. This occurred in many of the animals which had received injections of rabbit kidney on which streptococci had been grown (organisms killed with ether) and also in a few which had received plain mixtures of killed streptococci and rabbit kidney. The amyloidosis did not seem to coincide with high titers of antikidney antibodies.

The kidneys of other rabbits showed more or less pronounced pyelonephritis.

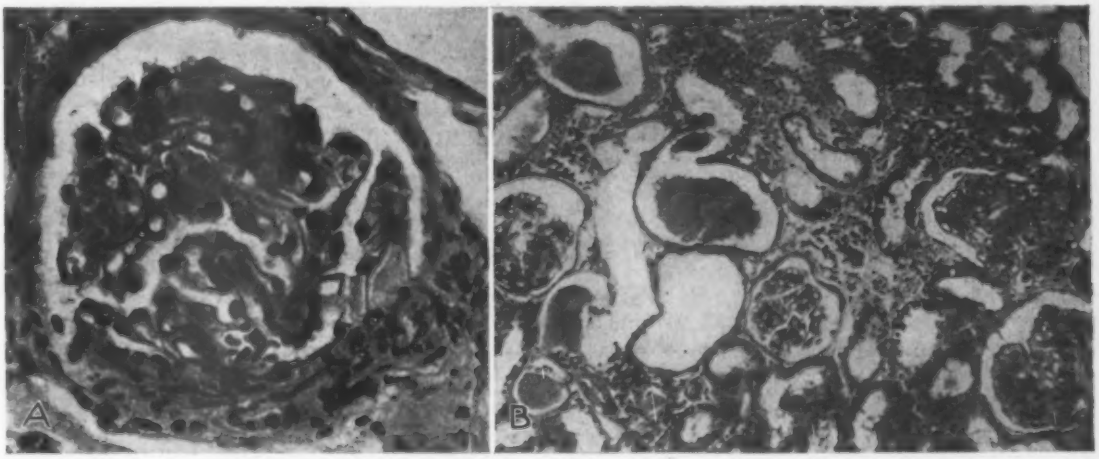

Fig. 3.—*A*, same as figure 2 *C*. Proliferative reaction of Bowman's capsule (× 370). *B* (rat 45), early chronic stage. Duration one hundred and ninety-four days. Glomeruli in various stages of hyalinization. Extensive capsular adhesions. Dilated tubules containing casts. Increase of the interstitial connective tissue and interstitial round cell infiltration (× 135).

COMMENT

Diffuse glomerulonephritis has been produced in rats by means of autoantibodies to kidney which had been formed in the animals themselves against their own kidneys on immunization with streptococcus-kidney antigen. Characteristically, the onset of the nephritis occurred about one to two weeks after the completion of the immunization, i. e., at a time when the production of antikidney antibodies could be expected to have reached its peak.

Several stages of the course of the disease have been observed. The majority of the rats recov-

2. It might be mentioned that in many rats several acute renal lesions were obtained by means of repeated immunizations; however, for the grouping used here, only 1 lesion per rat was considered.

3. Cavelti, P. A., and Cavelti, E. S.: Studies on the Pathogenesis of Glomerulonephritis: I. Production of Autoantibodies to Kidney in Experimental Animals, Arch. Path. **39**:148, 1945.

TABLE 3.—*Tabulation of the Pathologic Observations in Some of the Nephritic Rats*

Identification Number of Rat	Swelling of the Cells of the Glomerular Tuft	Proliferation of Endothelial and Epithelial Cells of the Tuft	Leukocytes in Glomerular Capillaries	Thickening of Glomerular Basement Membrane	Hyalinization of Tuft	Capsular Adhesions	Capsular Proliferation	Tubular Degeneration	Tubular Atrophy	Casts in Tubules	Proliferation of Interstitial Connective Tissue	Interstitial Infiltration (Lymphocytes)	Kidney Weight, per Cent of Normal	Heart Weight, per Cent of Normal	Proteinuria	Cylindruria	Hematuria (Microscopic)	Approximate Duration *	Interval Between Beginning of Immunization and Onset †	Interval Between End of Immunization and Onset ‡
316	+	+	+	0	0	0	0	+	0	0	0	+	105	..	+	(+)	0	1	14	7
464	+++	+++	++	0	0	0	+	+	0	0	0	+	125	88	+	+++	++	2	8	8
16	++	+++	+	0	0	0	+	0	0	0	0	+	129	..	+	++	+	2	7	4
332	++++	+	++	0	0	(+)	(+)	+	0	(+)	0	+	108	..	++	++++	++	3	13	6
488	++	+	++	0	0	0	0	0	0	++	0	+	158	132		Anuria		4	8	8
324	++	++	++	0	0	+	+	+	0	0	0	+	123	82	+	++++	++	6	11	4
309	+	++	+	±	0	+	(+)	++	0	0	0	++	124	85	(+)	++++	+	6	17	9
467	++	+	+	0	0	0	0	±	0	0	0	+	120	127	+	+++	+	7	8	8
203	++	++	+	±	0	+	(+)	+	0	+	0	+	144	116	+	+++	++	10	17	10
73	±	++	++	+	+	++	+	+	+	+	(+)	+	152	127	++	+++	++	20	12	7
39	±	++++	(+)	++	+	++	++	+	+	++	(+)	++	222	128	+++	+	±	68	11	5
25	0	+	(+)	+	—	(+)	—	++	+	+	+	+	189	123	+	+	(+)	70	16	3
49	0	++	+	++	++	++	+	±	+	++	(+)	+	172	155	++	++	+++	103	15	6
24	0	+++	+	++	+	++	+	+	+	++	+	+	143	84	++++	+++	(+)	137	18	5
59	0	++	(+)	++++	++++	+++	+++	+++	++++	+++	++++	+++	191	238	+++++	+++	+++	140	47	36
1	0	++	+	+++	++	+	±	++	++	++	++	++	147	122	++++	+++	+	157	8	5
45	0	+++	(+)	+++	+++	+++	++	+	++	++	+	++	177	124	+++++	+++	++	194	15	6
71	0	++	+	+++	++	+	(+)	+	+	+	(+)	+	155	181	+++	+	(+)	302	17	17
27	0	+	(+)	+	—	+	—	++	+	++	(+)	++	162	145	+++	++	+++	303	25	14
2	0	+++	++	++	—	+	+	++	+	+	+	++	199	113	++	++	++	341	28	12
23	0	++	+	+++	++	+	+	+	++	++	++	++	162	127	+++	+++	+	368	17	6

* The duration is taken as the number of days from the onset of the urinary abnormalities to death. The figures are not absolutely correct, as in most cases the urine was not examined every day.

† The number of days between the first injection of the schedule of immunization which had led to nephritis and the onset of urinary abnormalities. The figures are not absolutely correct, as in most cases the urine was not examined every day.

‡ The number of days between the last of the immunizing injections and the onset of the urinary abnormalities. The figures are not absolutely correct, as in most cases the urine was not examined every day.

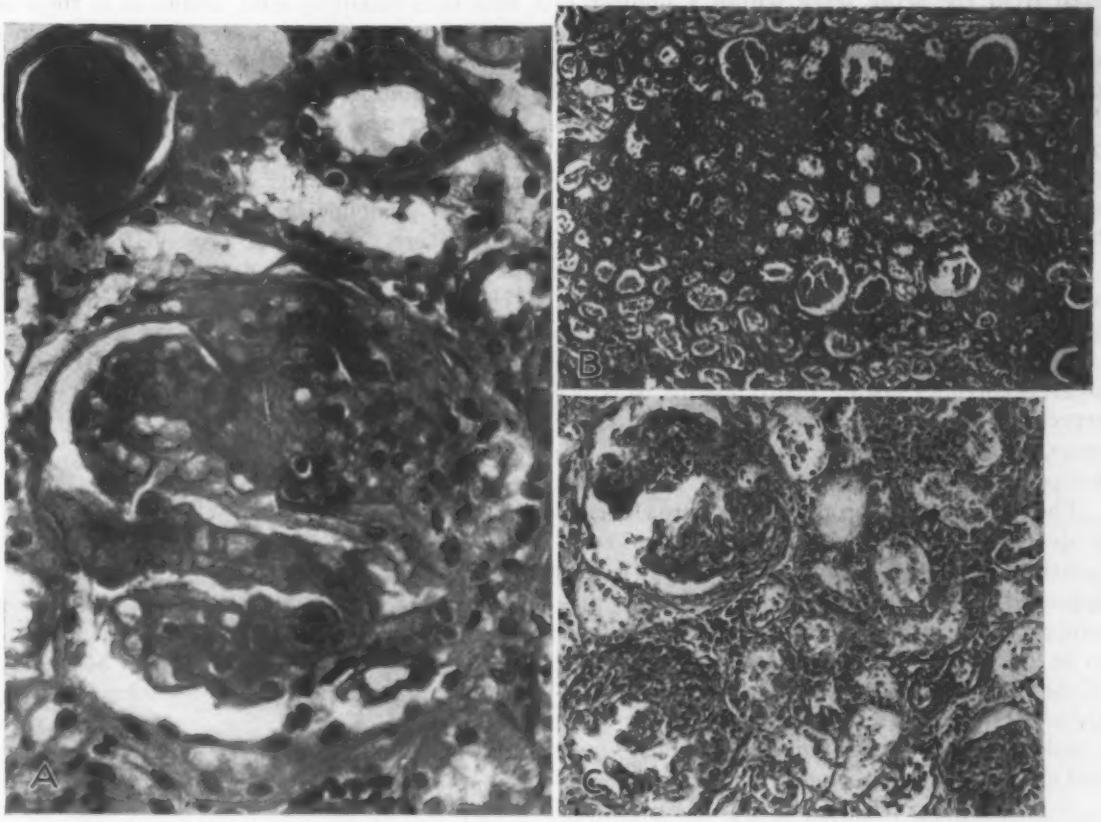

Fig. 4.—*A*, same as figure 3 *B* (× 500). *B* (rat 59), advanced chronic stage. Duration one hundred and forty days. Various stages of hyalinization of glomeruli. Capsular adhesions. Dilated and cystic tubules filled with casts. Extensive tubular atrophy. Marked increase of the interstitial connective tissue which is infiltrated with round cells (× 48). *C*, same as *B* (× 175).

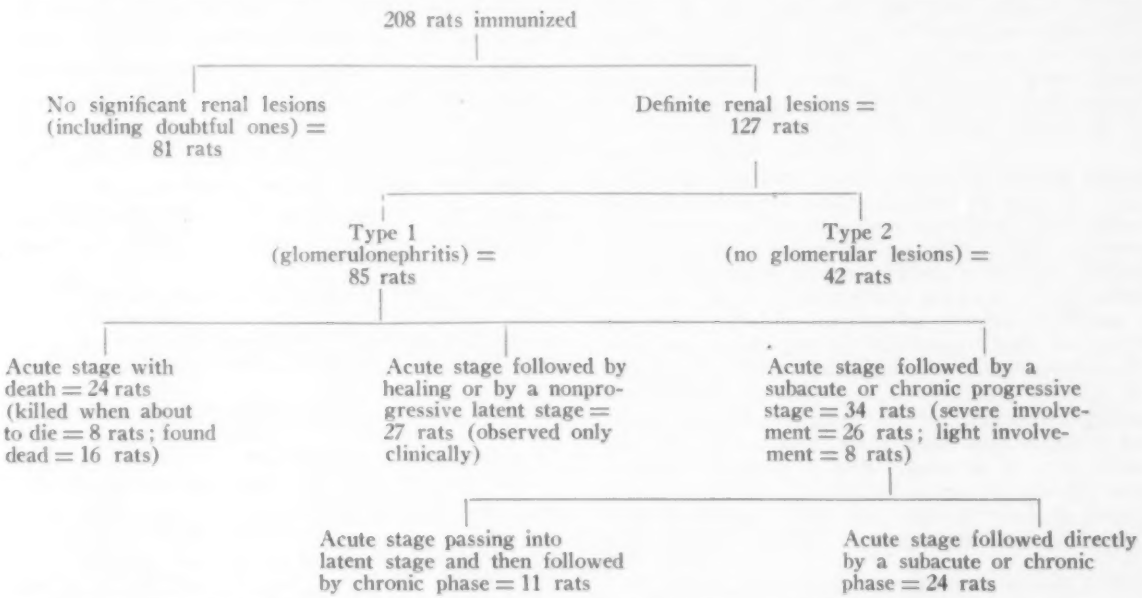

Fig. 5.—Diagram of a classification of the immunized rats with respect to the types of nephritis observed.

ered from the acute stage within a number of weeks. Another proportion of the animals died during this stage or reached a state of extreme emaciation that necessitated their being killed. Many of these rats had elevated levels of blood urea. Unfortunately, no blood for determinations of urea was available from some of those which died spontaneously and which seemed to have had the most severe lesions.

In still another proportion of the animals the acute was followed by a subacute and chronic stage.

Besides these stages of the disease, a latent stage, sometimes lasting many months, was observed. In a number of rats, after such a latent stage, active subacute or chronic lesions developed.

The subacute and chronic phases were clinically evident particularly in persisting and steadily increasing proteinuria. The severity of the lesions appeared to parallel roughly the degree of proteinuria, whereas the sedimentary findings seemed to be less reliable for the evaluation of the degree of the glomerular lesions. A number of the rats showing these phases when they died or were killed because of emaciation were found to have had elevated levels of blood urea.

The maximum duration of the chronic phase was about twelve months from the onset to death. The lesions of the rats which remained alive for such a long period, however, were observed to have been relatively mild, and most of these animals did not seem to have died of renal insufficiency. The more severe renal lesions led to death within about three to six months. The progressiveness of the lesions thus showed great variation. For reasons as yet not well understood, most of the rats with renal lesions did not survive—anatomically speaking—beyond the subacute stage. Such rats presented anatomically large, white kidneys. In this stage there was relatively little replacement fibrosis except in the glomeruli. However, there were a few rats in which the disease did progress further, so that replacement fibrosis was widespread and, in 1 rat, extremely marked; thus there was presented definite chronic nephritis.

SUMMARY

From the pathologic point of view the experimental glomerulonephritis produced in rats by means of autoantibodies to kidney consists essentially in exudative and diffuse proliferative processes involving the glomeruli, followed in severe forms by progressive obliteration of these structures.

Clinically the lesion is manifested by proteinuria, cylindruria and microscopic hematuria. Other clinical symptoms observed were edema, ascites, hypoproteinemia, lipemia, anemia, increase of blood urea and—as evidenced by cardiac hypertrophy—hypertension.

MORPHOLOGIC STUDIES OF RATS DEPRIVED OF ESSENTIAL AMINO ACIDS

II. LEUCINE

MARK E. MAUN, M.D.; WILLIAM M. CAHILL, M.D., AND RUTH M. DAVIS, B.A.*

DETROIT

In a recent study we[1] reported observations on rats fed a synthetic diet completely devoid of the amino acid phenylalanine. The animals lost weight, their hair grew coarse and unkempt, and they became progressively weaker throughout the experiment. The deficient rats when compared with the control animals, which were maintained on an isocaloric but complete diet, showed: (1) reduction of hemoglobin and plasma proteins; (2) narrowing of epiphysial cartilages of the long bones; (3) marked thymic atrophy; (4) atrophy of the cortices of the adrenal glands, with a decrease of lipoid content; (5) degeneration and atrophy of the epithelium of the seminiferous tubules of the testes.

Other data regarding the effects of amino acid deficiencies have been presented by several observers. Rats fed lysine-deficient diets failed to gain in weight and became progressively weaker, and when they were killed, reduction of hemoglobin and plasma proteins was found.[2] The long bones and the testes showed alterations similar to those noted with diets devoid of phenylalanine. Consumption of tryptophan-deficient diets by rats resulted in loss of fertility,[3] and several observers have reported cataract formation when these animals were fed such rations for a prolonged period.[4] A deficiency of lysine or tryptophan in purified diets has been observed to result in corneal lesions similar to those in animals with riboflavin deficiency.[5] The development of corneal changes in rats on tryptophan-deficient rations was detected by slit lamp observation and confirmed with histologic preparations. Vascularization of the cornea appeared in some animals that had been fed the diet about ten days; subsequently the cornea became dry and cloudy, apparently owing to epithelial changes and leukocytic infiltration. These alterations could be removed by addition of tryptophan to the rat's diet, but the formed cataracts were permanent. The significance of corneal vascularization is not yet clear, since it has been observed following numerous experimental procedures.

In the experiment recorded here, a group of rats of a single strain of the same age and comparable weights was fed a purified diet in which the nitrogen was supplied by a mixture of crystalline essential amino acids. Some of the animals received all of the essential amino acids, while others received all the essential amino acids except leucine.

EXPERIMENTAL PROCEDURE

Diets.—The diets consisted of a synthetic mixture similar to that previously described by us.[1] In the present experiment the control ration contained 25.6 Gm. of leucine per kilogram, whereas in the leucine-deficient diet that amount of leucine was replaced isocalorically with sucrose. The diets were supplemented with crystalline vitamins.[6] The average daily food intake was 3.14 Gm. per rat.

Animals.—In the present experiment weanling rats of the Fisher line 344 strain, 25 days old, were pair fed. The animals on the two diets were kept in individual cages, and coprophagy was prevented. The rats were fed the respective diets for twenty-eight days, and all survived. At the termination of the experimental period they were killed by decapitation without anesthesia, a procedure which proved satisfactory with regard to obtaining blood for hemoglobin and plasma protein determinations.

Autopsies.—Immediately after the animals were killed, autopsies were made. Each organ was inspected and weighed. Portions of each organ (or an entire organ if there were two of a kind—e. g., adrenal glands) were fixed in Zenker's fluid and other portions in Bouin's fluid. Of the two of a kind, one was fixed in Zenker's

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1. Maun, M. E.; Cahill, W. M., and Davis, R. M.: Arch. Path. **39**:294, 1945.

2. Harris, H. A.; Neuberger, A., and Sanger, F.: Biochem. J. **37**:508, 1943.

3. Albanese, A. A.; Randall, R. M., and Holt, L. E., Jr.: Science **97**:312, 1943.

4. Busehke, W.: Arch. Ophth. **30**:735, 1943. Totter, J. R., and Day, P. L.: J. Nutrition **24**:159, 1942.

5. Bessey, O. A., and Wolbach, S. B.: J. Exper. Med. **69**:1, 1939.

6. All the amino acids and vitamins required in these experiments were supplied by Frederick Stearns & Company,

and the other in Bouin's solution. Additional portions of organs were placed in 4 per cent solution of formaldehyde. Sections of the long bones, the sternum and the vertebrae were decalcified in Zenker's fluid. The tissues were embedded in paraffin, sectioned at 6 microns and stained routinely with hematoxylin and eosin. Additional stains were employed as necessity demanded. After fixation in Zenker's fluid, one of the eyes was embedded in paraffin, the other in celloidin (a concentrated preparation of pyroxylin), in each instance.

RESULTS

Animal Health.—The animals fed the leucine-deficient diet became weak, had difficulty in walking and were relatively inactive. These rats would not keep themselves clean, and their hair became thin and dull as contrasted with the heavy, glossy hair of the control animals.

Body Weight.—The food consumption of the deficient group was identical with that of the control group (3.14 Gm. per day), but the rats on the deficient diet lost weight throughout the entire experimental period. The average loss of weight of the latter animals was 17.4 Gm., while that of the control group averaged only 3.1 Gm. The loss of weight of the control animals can be attributed to undernourishment, since in this paired feeding experiment their food consumption was limited to that of the deficient animals (fig. 1).

Roentgen Studies.—Roentgenograms of the complete skeletons of both the control and the deficient group were obtained three days prior to the end of the experimental period. At this time no alterations could be discerned.

Organ Weights.—Each organ was weighed immediately after its removal, and the ratio $\frac{\text{organ weight}}{\text{body weight}}$ was determined. These weights were compared in each experiment, and the average weights were submitted to statistical analysis. Significant alteration of weight was noted in the liver, the spleen, the thymus and the pituitary gland of the deficient animal. The pituitary gland showed definite hypertrophy, but the other organs were atrophic.

Hemoglobin.—Determinations of hemoglobin were made by the Sheard-Sanford method.[7] There were, however, no significant alterations, the average hemoglobin content of the blood of the deficient animals being 13.9 Gm. per hundred cubic centimeters, whereas that of the control animals was 13.26 Gm.

Plasma Protein.—The plasma protein, determined by the micro-Kjeldahl method, showed no significant alterations. The average value for the plasma protein of the deficient animals proved to be 5.25 Gm. per hundred cubic centimeters, compared with 5.21 Gm. for the control animals.

Liver Fat.—The fat content of the liver showed slight alterations, which were not considered to be significant. It was determined by the method of Leathes and Raper.[8] The average content for the deficient animals was 5.06 Gm. per hundred grams of liver, whereas the average for the control group was 4.72 Gm.

MORPHOLOGIC OBSERVATIONS

Liver.—The livers of the deficient animals were normal on gross inspection but proved to be smaller,

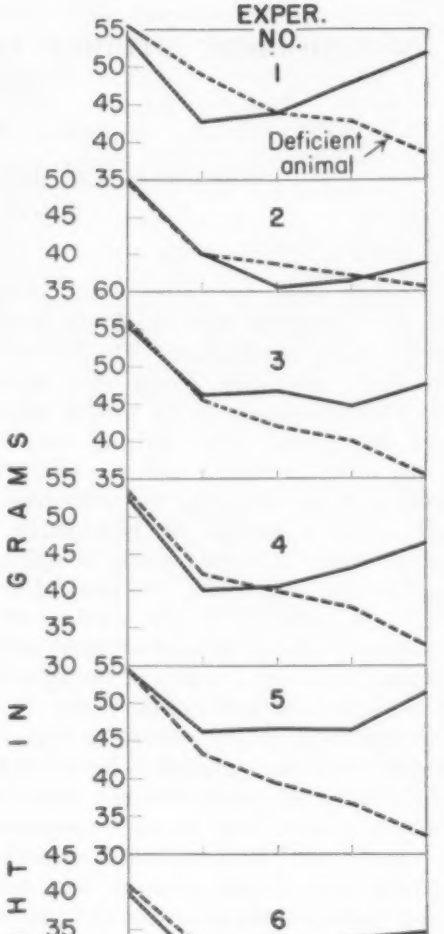

Fig. 1.—Weight curves of the control and the leucine-deficient animal in nine paired feeding experiments.

7. Sheard, C., and Sanford, A. H.: J. Lab. & Clin. Med. **14**:558, 1929.

8. Leathes, J. B., and Raper, H. S.: The Fats, ed. 2, New York, Longmans, Green & Co., 1925.

making up 3.863 per cent of the body weight, compared with 4.221 per cent for the control rats. The average weight of the liver of deficient animals was 2,101 ± 48 mg., compared with 1,629 ± 56 mg. for the control animals. Portions were fixed in Zenker's fluid, alcohol and Bouin's fluid. Among the prepared sections of liver, the hematoxylin-eosin sections of 2 of the deficient rats showed a narrow zone about the central veins in which hepatic cells appeared to contain small vacuoles. Frozen sections of these areas stained readily with sudan III, indicating the presence of lipoids. The remaining portion of each lobule presented no alterations. With Best's carmine the hepatic cells of the leucine-deficient animals contained less stainable glycogen, but these differences were neither constant nor marked in degree.

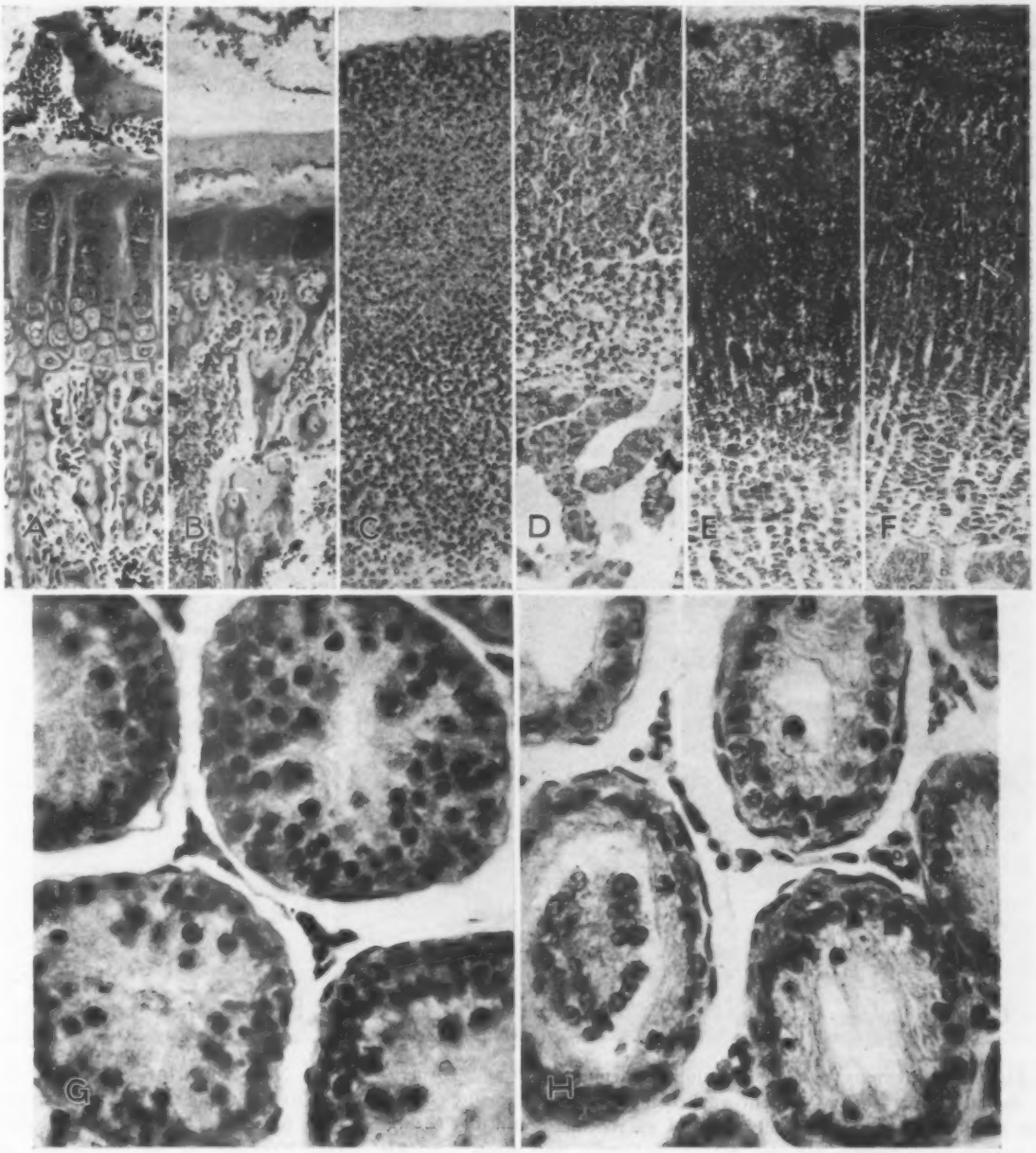

Fig. 2.—*A* and *B*, sections showing epiphysial lines of a control and a leucine-deficient rat; hematoxylin and eosin; × 138. *C* and *D* sections of adrenal glands of a control and a leucine-deficient animal; hematoxylin and eosin; × 138. In *D* note thinness of cortex and prominence of sinuses. *E* and *F*, sections of adrenal glands of a control and a leucine-deficient animal; sudan IV; × 138. In *F* note decreased lipoid content. *G* and *H*, sections of testes from a control and a leucine-deficient rat; hematoxylin and eosin; × 322. In *H* note degenerative changes and decreased number of mitoses.

Spleen.—The spleens from the deficient animals were atrophic, weighing 0.329 per cent of the body weight, compared with 0.430 per cent for the control rats. The average weight of the spleens of the deficient rats was

108 ± 11 mg., compared with 186 ± 18 mg. for the control group. Examination of the prepared sections revealed the structure of the organ to be well preserved in both groups, but the lymphoid follicles in the spleens of the deficient rats were small, and the germinal centers were absent. In the spleens of the deficient animals, multinucleated giant cells were frequently seen adjacent to the lymphoid follicles.

Thymus and Lymph Nodes.—Marked thymic atrophy was apparent in the leucine-deficient animals on inspection of the open thorax; the glands weighed but 0.034 per cent of the body weight, compared with 0.138 per cent for the control animals, the average weight of the thymus in the deficient rats being 11 ± .97 mg., compared with an average weight of 64 ± 4.9 mg. for the control animals. Examination of the prepared sections of the glands from deficient rats revealed the lobular pattern to be accentuated by a marked increase of intralobular connective tissue. The medullary and cortical zones were difficult to identify, because of a marked decrease in the lymphoid elements and an apparent increase in connective tissue. The latter proved to be a relative increase, due to the almost complete disappearance of lymphocytes and to fibroblastic proliferation with production of newly formed collagenous fibers. This was most marked in the medullary portions of the gland.

The lymph sinuses were prominent, and on close examination they were seen to be lined by large rounded cells with a faintly staining eosinophilic cytoplasm containing many small vacuoles. Fusion of these phagocytes was often apparent, and the resultant cell mass formed multinucleated giant cells. In the newly formed stroma, irregular clefts were seen; these were surrounded by giant cells and large lymphocytes.

Bones and Joints; Bone Marrow.—Prepared sections of the long bones of the deficient rats showed narrowing of the epiphyses as compared with those of the control animals. The thinning resulted from a decrease in number of chondroblasts and was similar to that noted in phenylalanine deficiency. There was also an evident

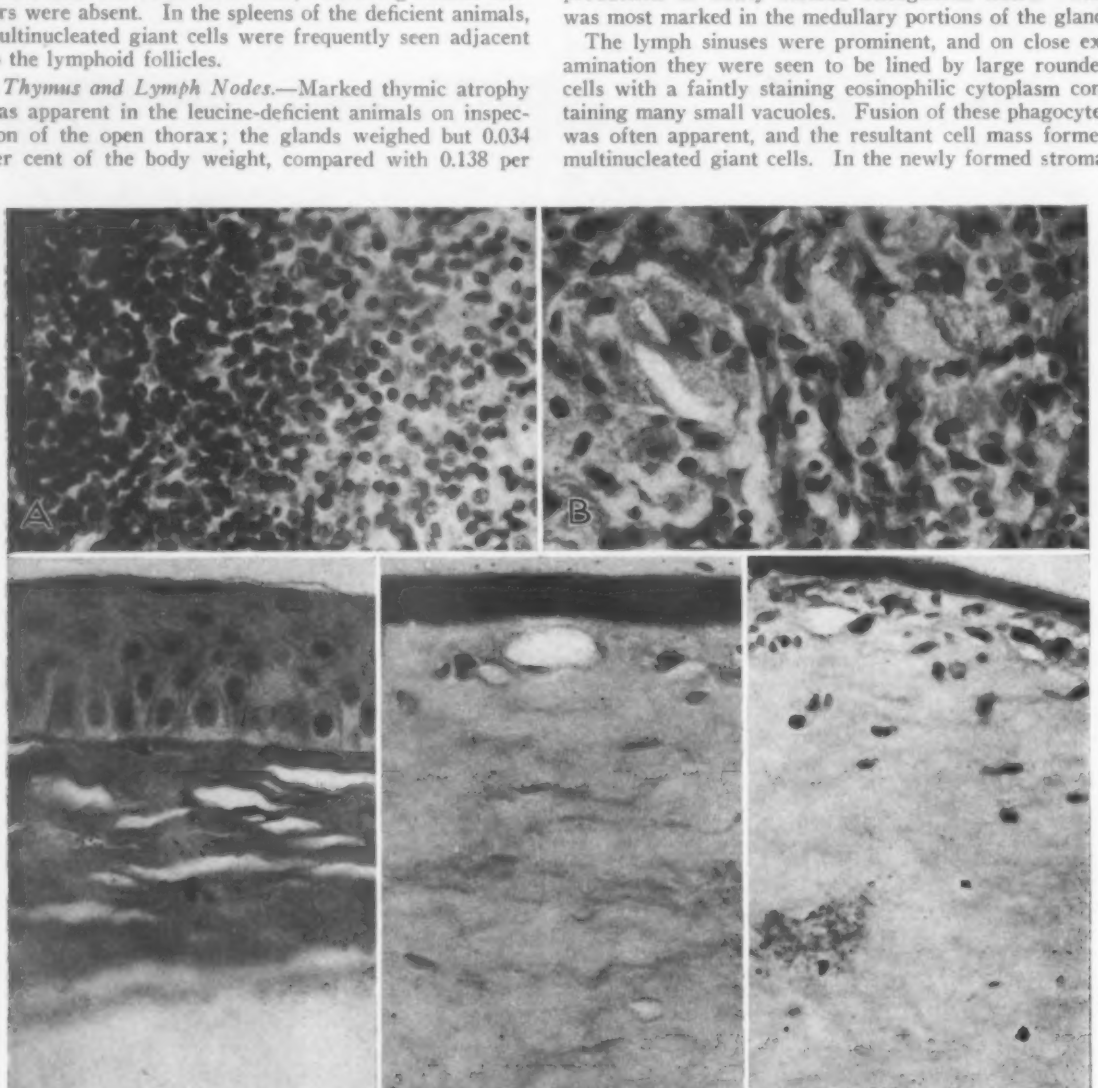

Fig. 3.—*A*, thymus of a control animal; hematoxylin and eosin; × 350. Note abundant lymphoid tissue. *B*, thymus of a leucine-deficient rat; hematoxylin and eosin; × 350. Note sparse lymphoid tissue and prominent phagocytes. *C*, cornea of a control rat; hematoxylin and eosin; × 400. Note structure of the epithelium. *D*, cornea of a leucine-deficient rat; hematoxylin and eosin; × 400. Note thinning of the epithelium and prominent capillaries in the substantia propria. Compare the structure of the latter with the normal. *E*, cornea of a deficient rat showing more severe damage; hematoxylin and eosin; × 400. Note leukocytic infiltration of the substantia propria and of the anterior chamber.

sparsity of trabeculae in the deficient animals, but the degree of calcification of the bones was equal in animals of both groups.

The bone marrows of the control animals and those of the deficient rats showed no differences.

Adrenal Glands.—In weight the glands from the leucine-deficient rats were almost comparable to those from the control rats. One gland from each animal was fixed in Zenker's fluid. The other was placed in Bouin's fluid, and after fixation the gland was bisected. From one half frozen sections were stained with sudan IV and from the other half and from the gland fixed in Zenker's fluid sections were prepared and stained with hematoxylin and eosin. Examination of the glands of the deficient animals showed the medullary portions to be unaltered, but the cortices appeared thinned, with "compression" of the middle zones. More careful examination disclosed narrowing of both the zona glomerulosa and the middle zone, whereas the inner zone was narrowed in only a few instances. More striking were the prominent sinuses that widely separated the thin cords of the zona reticularis. These prominent sinuses could be easily traced into the outer portions of the middle zone. Examination of the cells forming the different zones revealed some nuclei of the zona glomerulosa to be small and pyknotic. Cells of the outer portion of the fascicular zone presented a similar appearance, but abnormal mitoses were rare. The cells of the outer and middle zones appeared less prominent, while those of the zona reticularis appeared small and atrophic as compared with those of the control animals.

The adrenal glands subjected to fat stains presented more striking changes. The glands of the control animals showed abundant lipoids in the middle zone, with small quantities in all cells of the inner zone and in some cells of the zona glomerulosa. Glands of the leucine-deficient animals showed the inner zone to be almost free of lipoids and the outer portion of the middle zone to contain only small amounts. The decrease in cortical lipoids and the thinning of the cortices of the adrenal glands simulate the atrophy previously noted in experimental starvation (Jackson [9]) and that reported by us in phenylalanine deficiency.

Male Genital System.—The testes from rats on the leucine-deficient diet were somewhat smaller by weight than those from the control group, but this alteration did not prove to be significant, and both those from the control and those from the deficient animals were softer than usual. One testis from each animal was fixed in Zenker's fluid and the other in Bouin's fluid. Examination of the prepared sections showed in the testes of the leucine-deficient animals changes limited to the tubular epithelium. The Sertoli cells were prominent, owing to a decreased number of mitoses and to failure of the epithelial cells to form primary spermatocytes except in rare instances. A few degenerative cells were found within the lumens of the tubules. In the control group the prostate and the epididymis showed no changes. The testes of this group revealed early retrogressive changes of the tubular epithelium which were less pronounced but similar to those of the deficient animals. An explanation of the latter changes is not readily apparent. They are possibly to be attributed to malnutrition, since the caloric intake of the control animals was determined by that of the leucine-deficient rats.

Pituitary Gland.—Inspection of the pituitary glands of the deficient rats revealed them to be visibly larger than those of the control animals. The glands of four pairs of animals (experiments 6, 7, 8 and 9) were weighed, and those of the deficient rats were found to be significantly larger, forming 0.019 per cent of the body weight, compared with 0.008 per cent for the control group. The mean weight of the glands of the control animals was 3.25± 0.084 mg., compared with 7.25 ± 98 mg. in the deficient animals. The glands were sectioned at 4 microns and stained with hematoxylin and eosin and with azocarmine.

The prepared sections showed no cellular alterations in the glands from either group, and differential counts of the stained cells revealed the cell distribution to be similar.

Eyes.—The eyes were not observed with unusual care during the experimental period, but on casual inspection corneal ulcers were noted in 2 rats of the leucine-deficient group. One ulcer appeared during the first ten days of the feeding period and was attributed to an injury. The prepared sections were made at 5 microns and were stained with hematoxylin and eosin and Mallory's trichrome stain. The eyes of the control animals appeared to be normal.

In the deficient group, all the eyes showed variable alterations, those in the cornea being most pronounced. The epithelium of the cornea was thinner, and often there was wrinkling of the surface. The cells of the thinned epithelium showed a loss of polarity to form several flattened layers with production of keratin on the surface. The basal cells of the epithelium showed an increased number of mitoses and stained more deeply with hematoxylin. The substantia propria was thickened and homogeneous, as the fibrillar structure was lost. Blood vessels beneath the epithelium and those in the superficial portion of the substantia propria were dilated and prominent; about them and in the adjacent stroma small collections of granulocytes were present. Descemet's membrane appeared to be slightly thickened. The vascularization and the epithelial metaplasia of the cornea were more evident near the limbus.

More pronounced lesions consisted of congestion and leukocytic infiltration of the iris and the ciliary body. In several instances the anterior chamber contained fibrin and collections of leukocytes. Present with these lesions were vesiculation and leukocytic infiltration of the corneal epithelium. No alterations of the lens or of the posterior portion of the eye were observed.

Other Organs and Tissues.—Certain organs and tissues were inspected and, when feasible, weighed. Sections were prepared from each for histologic examination. The methods employed have been previously described. This group included the heart, blood vessels, the lungs, the pancreas, the kidneys, voluntary muscle, skin, the urinary bladder, the salivary glands, the thyroid and parathyroid glands, the female genital system and the central and peripheral nervous system. These tissues and organs of the leucine-deficient animals were comparable with those of the control animals.

COMMENT

The clinical condition of the leucine-deficient animals was similar to that of animals fed diets devoid of phenylalanine in that the rats lost weight throughout the experiment and became gradually weaker. However, the leucine-deficient animals failed to show a loss of hemoglobin or plasma protein. The changes in the tissues of the leucine-deficient animals were similar save for (1) apparent hypertrophy of the pituitary gland, (2) vascularization and metaplasia of the

9. Jackson, C. M.: Am. J. Anat. **25**:221, 1919.

corneal epithelium and, in some instances, congestion and leukocytic infiltration of the ciliary body and iris. It is noteworthy that comparable ocular changes have been previously observed by other investigators in deficiencies of lysine, or tryptophan and of riboflavin.

SUMMARY

Young rats of a single strain were fed synthetic diets in which the nitrogen was supplied by crystalline amino acids. They were divided into a control and a deficient group. The control group continued to receive all of the essential acids; leucine was removed from the diet of the deficient group. The animals were pair fed so that the food consumption was equal in both the control and the deficient group. During the experimental period the leucine-deficient animals lost weight and became progressively weaker. The rats were killed at the end of the twenty-eight day experimental period, and autopsies were done. The leucine-deficient animals showed moderate atrophy of the liver and the spleen; profound atrophy of the thymus; atrophy of the adrenal cortex with a decrease in the lipoid content; hypertrophy of the pituitary gland; vascularization and leukocytic infiltration of the cornea, and atrophy of the epithelial cells of the seminiferous tubules.

Case Reports

RHABDOMYOSARCOMA

HENRY R. VIETS, M.D., AND MARTIN H. WITTENBORG, M.D., BOSTON

Rhabdomyosarcoma belongs to a group of tumors many of whose aspects are still unfamiliar. The method of growth and of recurrence and the unusual neurologic complications in the case reported here are considered of interest.

SUMMARY OF THE CLINICAL RECORD

C. H., a man aged 49, was first seen Sept. 10, 1940 with cellulitis of the right leg, a condition which responded rapidly to treatment.

Sept. 10, 1942 the patient was seen again with a history that three months earlier his wife had noticed a painless swelling on his back. No similar nodule had ever been noted elsewhere on the body, and there was no history of cancer in the family. The growth had extended in a period of three months so that on examination it was 9 by 7 cm. in diameter. Firm, nontender, appearing like an encapsulated tumor beneath the right shoulder blade, it was not attached to the skin but was firmly attached to the latissimus dorsi muscle. Roentgenograms of the spine taken at the level of the tumor failed to show any deviation from the normal. The growth arose from the sacrospinalis muscle and when excised appeared to be cancerous in type, either fibrosarcoma or rhabdomyosarcoma. The deep fascia in the immediately underlying muscles were removed in a block dissection. Roentgenograms of the ribs and the chest failed to disclose any signs of metastases. There was no evidence of involvement of the nervous system. The diagnosis from examination of the tissue removed was spindle cell sarcoma. The patient left the hospital September 30.

He returned to the hospital November 15, with severe pain beneath the scar of the operation. There was no evidence of recurrence of the tumor. The patient took large and frequent doses of codeine. A bilateral Babinski sign, absence of the left ankle jerk, a reduced left knee jerk and atrophy of the left quadriceps muscle were noted. No change in sensation was detected. The next day hyperesthesia was found over the area supplied by the right eleventh and twelfth thoracic nerve roots, with tenderness over the spines of the eighth and ninth vertebrae on percussion. Control of micturition was normal. Roentgenograms of the thoracic and lumbar portions of the spine and of the chest showed no evidence of metastases.

By March 1943 the patient had lost 15 pounds (6.8 Kg.) in weight. There was no change in the neurologic signs. Roentgen study of the chest revealed numerous round shadows scattered through both lung fields. A diagnosis of extensive pulmonary metastases was made. In April 1943 his pain increased severely, and he was given small doses of roentgen radiation, 300 roentgens to the tumor area on each of four occasions. He received considerable benefit from the roentgen treatment and was free from pain for nearly a month. The pain then recurred, and the patient began to cough. A roentgenogram, May 25, 1943, showed a large tumor mass on the right side of the chest and extensive metastases in both lung fields. Roentgen treatment was given again in June 1943, 400 roentgens in each of four treatments. In July he had mild hemoptysis and continued to show signs of advancing disease. This was confirmed by subsequent roentgenograms of the chest in July 1943.

In August 1943 there developed, within a few hours, signs of complete dysfunction of the spinal cord with retention of urine, paralysis, and loss of sensation up to the level of the tenth thoracic segment of the spine. He died Aug. 13, 1943.

Autopsy (three and one-third hours post mortem by Dr. J. Tullis).—The body was that of a moderately emaciated white man, 177 cm. in length and weighing 54 Kg. Along the vertebral border of the right scapula was a well healed transverse surgical incision at the level of the fifth thoracic segment of the spine.

Originating from the muscles of the back was a soft, spherical, nonencapsulated mass measuring 6 cm. in diameter, which eroded the posterior segments of the sixth through the tenth right ribs and vertebral bodies and compressed the midposterior portion of the right lung without penetrating the pleura. It was continuous with numerous clearly demarcated masses of identical character within the spinal canal extending from the sixth to the eleventh thoracic vertebral bodies, compressing the spinal cord but entirely extradural. On cut section the tumors presented a yellow, soft, partly necrotic surface, trabeculated with thin fibrous septums.

The pleural cavities were obliterated bilaterally by thin fibrous adhesions. Both lungs revealed numerous spheroid masses, clearly demarcated and of varying size up to 4 cm. in diameter of identical character without involvement of bronchi. The right kidney contained an isolated, firm, nonencapsulated, clearly demarcated spheroid mass protruding slightly from the anterior surface and on section identical with the tumor found elsewhere.

Microscopically, these masses were of the same character. The tumor was nonencapsulated, densely cellular and highly invasive, invading lymphatics, blood vessels, bone, soft tissue and dura. It grew in interlacing strands, without a fibrous stroma, and presented numerous foci of hemorrhage and necrosis. The cells were characterized by marked pleomorphism and considerable variation in size (fig. 1). The larger cells were 100 microns or more in length and 36 to 40 microns in width. They varied from small, irregular spindle-shaped cells with single fusiform nuclei to large, greatly elongated, strandlike, ribbon-like or droplet-shaped cells, rich in cytoplasm and frequently multinucleated. The cytoplasm of these larger cells revealed fine longitudinal striation, and that of a few, transverse striation as well

From the Neurological Service of the Massachusetts General Hospital and the Laboratory of Pathology of the New England Deaconess Hospital.

(figs. 2 and 3). The nuclei were large, well outlined and predominantly fusiform, with blunt ends. The nucleoplasm revealed condensation along the nuclear membrane with a hyperchromatic granular pattern and one or more large spherical hyperchromatic nucleoli. In addition, there were numerous multinucleated round or irregular tumor giant cells, with sparse undifferentiated cytoplasm, containing up to ten nuclei each. Mitoses were frequent and often atypical.

Additional pathologic changes consisted of bronchopneumonia and cavitation of the spinal cord. The latter was interpreted as secondary to vascular compression by the tumor mass.

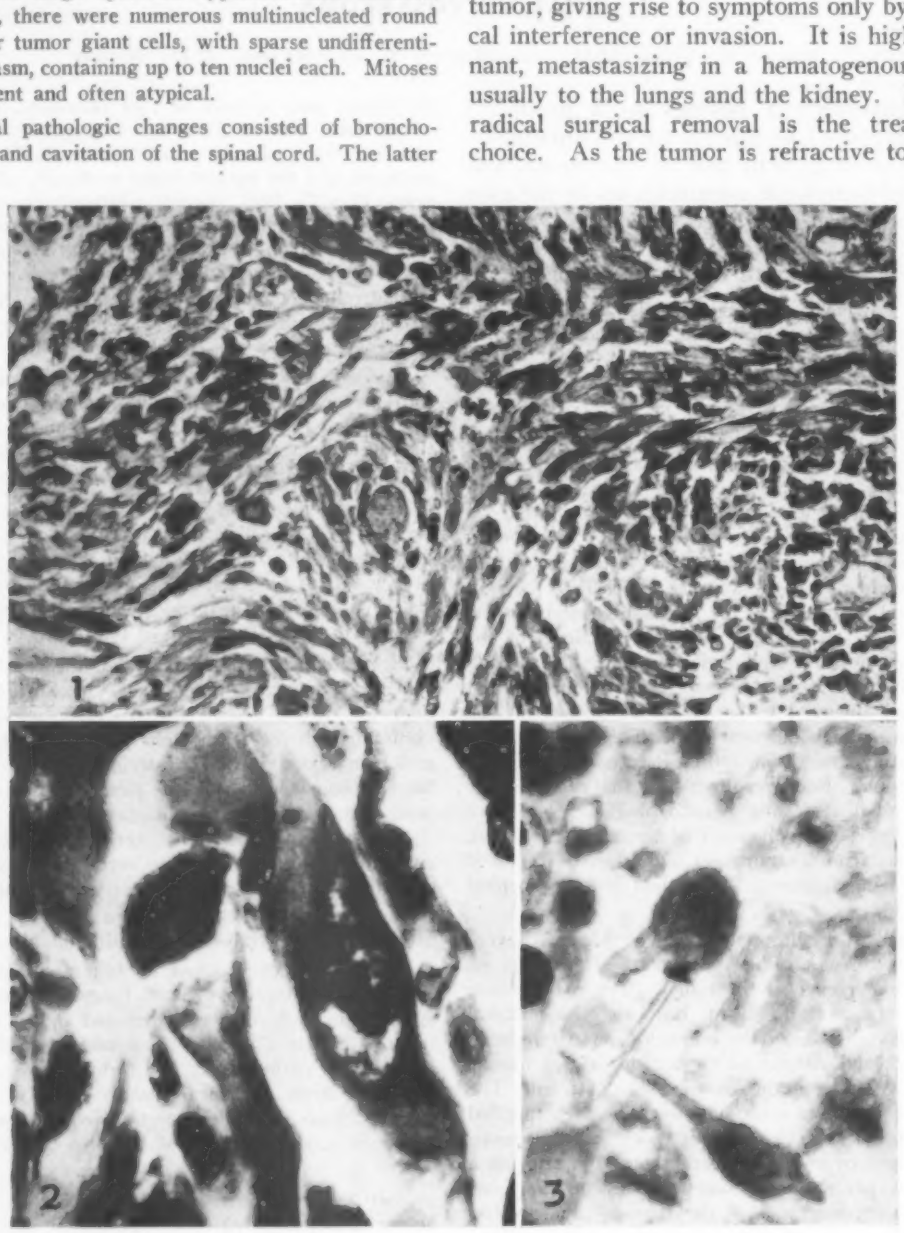

Fig. 1.—Area of the tumor showing the absence of stroma, the cellularity, the pleomorphism of the cells and the great length of some cells. Mallory's phosphotungstic acid-hematoxylin; × 160.

Fig. 2.—Detail of elongated giant cells showing suggestive striation. Note the multinucleated giant cell. Masson's trichrome stain; × 700.

Fig. 3.—Elongated cells showing striations. Mallory's phosphotungstic acid-hematoxylin; × 1,000.

COMMENT

Rhabdomyosarcoma is a neoplasm of that highly differentiated mesenchymal tissue the striated muscle. Despite the great mass of striated muscle in the body, it is an uncommon tumor. A survey of the literature indicates that the rhabdomyosarcoma of the voluntary muscles usually presents itself as a painless progressive tumor, giving rise to symptoms only by mechanical interference or invasion. It is highly malignant, metastasizing in a hematogenous pattern, usually to the lungs and the kidney. Early and radical surgical removal is the treatment of choice. As the tumor is refractive to roentgen therapy, postoperative recurrence is the rule. Statistically significant figures as to age and sex incidence or site of origin have not been formulated.

Microscopically, the tumor is characterized by nonepithelial cells, invasion, pleomorphism,

mitotic activity and strandlike or droplet-shaped cells showing transverse striation of the cytoplasm, similar to the striation of voluntary muscles. The pleomorphism is usually marked and the mitotic activity often atypical. The striations, though often difficult to demonstrate, are frequently the only distinctive characteristic to differentiate the rhabdomyosarcoma from other highly malignant, poorly differentiated sarcomas of the soft parts.

The so-called rhabdomyosarcoma found in the heart muscle is not cancerous and more common. The myoblastoma, representing the precursor of the muscle cells, does not present the characteristic striation and occurs frequently in the tongue.

SUMMARY

In the case described, confirmed by autopsy, rhabdomyosarcoma eroded the spinal column and compressed the spinal cord, resulting in neurologic complications. These complications are considered unusual for a sarcoma of the soft parts. Insufficient cases of rhabdomyosarcoma of the skeletal muscles have been reported to permit statistical clinical analysis of this pathologically well defined but uncommon tumor.

ADENOCARCINOMA OF THE JEJUNUM ASSOCIATED WITH HYPERPLASIA OF THE PARATHYROID GLANDS AND GENERALIZED OSTEOPOROSIS

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The enlargement of the parathyroid glands in osteitis fibrosa cystica and in other diseases of bones,[1] the presence of hyperplasia or adenoma of the parathyroid glands in hyperparathyroidism with or without associated osteitis, renal calculi or both,[2] the enlargement of the parathyroid glands in chronic renal disease [3] and the hyperplasia of the parathyroid glands in chronic renal insufficiency, particularly in chronic glomerulonephritis,[4] have been well described and fairly widely recognized. The association of hyperplasia of the parathyroid glands with diseases of the gastrointestinal tract has not been especially stressed in the reports available in the literature. Lloyd,[5] in describing a case of hypophysial tumor with associated tumor-like enlargement of the parathyroid glands and the pancreatic islets, briefly mentioned another case in which a patient of unstated age and sex was afflicted with severe tetany. Autopsy disclosed chronic nephritis, extreme pyloric stenosis and calcium deposits in the mucosa of the ileum. Also observed were five enlarged parathyroid glands, the largest measuring 9 by 5 by 4 mm., all showing many eosinophilic cells and numerous mitoses. The condition was thought to represent simple hyperplasia of the parathyroid tissue, for no structures resembling adenoma were found. Castleman and Mallory [4] listed 2 cases of duodenal ulcer. One of the patients had concomitant gastritis and pituitary basophilism. Both patients showed hyperplasia of the parathyroid glands, but the hyperplasia was not nearly so pronounced as that in their group of 12 patients with chronic glomerulonephritis. In a paper detailing the case of a 47 year old woman with hyperplasia of the parathyroid glands secondary to renal insufficiency caused by polycystic kidneys, Nelson [6] described also the case of a 73 year old woman in whom autopsy revealed multiple intestinal obstruction from metastases of a carcinoma of the gallbladder. The four parathyroid glands, which weighed 910 mg., histologically consisted of "only *wasserhelle* and transitional *wasserhelle* cells." The kidneys, the ribs and the vertebrae showed no pathologic changes either grossly or microscopically. Since the cases cited were the only instances of hyperplasia of the parathyroid glands occurring with lesions of the gastrointestinal tract found in the literature, the case which I shall report is of interest because of the presence of adenocarcinoma of the jejunum, hyperplasia of the parathyroid glands, adenoma of one parathyroid gland and generalized osteoporosis.

REPORT OF A CASE

A white woman employed as a housemaid was first admitted to the Colorado General Hospital on March 3, 1935 when she was 51 years old. At that time she was suffering from "generalized osteomalacia," questionably due to sensitivity to a gold compound that was being injected, and from fractures of the ulnas. For thirteen years before admission she had recurrent attacks of diarrhea at intervals of three or four months. Three years before, small, red, slightly raised spots on the left cheek became larger and coalescent. Lupus erythematosus, diagnosed elsewhere, was treated with injections of a gold salt. After the third injection, cervical, axillary and inguinal lymphadenopathy developed. Following the seventh injection, large black and blue areas were observed on the legs, with tarry stools and a blood hemoglobin content of 14 per cent. She was hospitalized elsewhere, received a transfusion and was discharged in eighteen days with a residuum of pain in the joints when in the erect posture. For nine months before admission to the Colorado General Hospital she experienced pain in the back and the hips. Four months before, she was seized with sudden pain in the forearms while in the act of lifting a small child. Fractures of the ulnas were seen in roentgenograms of the forearms, to which casts were applied. Examination on admission revealed a temperature of 98 F., a pulse rate of 68 per minute and a respiratory rate of 20 per minute. The blood pressure was 120 systolic and 80 diastolic. The significant physical findings were false dentures in situ, a nontransmitted systolic murmur over the apex of the heart, plaster casts in place over the forearms, a discolored ecchymosis over the right ankle and varicosities of the left foot. The urine contained no Bence Jones protein and was normal to the usual tests. The blood hemoglobin amounted to 12 Gm. per hundred cubic centimeters, the erythrocytes to 4,870,000 per cubic millimeter, the leukocytes to 4,600 to 9,500 per cubic millimeter, with a partition count of

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65 per cent polymorphonuclear neutrophils, 27 per cent lymphocytes, 6 per cent monocytes and 2 per cent eosinophils. The blood platelets numbered 320,000 per cubic millimeter, the coagulation time was five minutes, the bleeding time three minutes and the sedimentation rate 7 per cent in one hour. The blood serum revealed no Bence Jones protein. The blood showed calcium 9 mg., phosphorus 3 mg., nonprotein nitrogen 24 mg., total proteins 5.74 Gm., albumin 2.76 Gm. and globulin 2.98 Gm. per hundred cubic centimeters. The basal metabolic rate was plus 8. A biopsy of the sternal marrow was unsuccessful. Roentgen examination of the skull, the pelvis, the spine and the long bones disclosed pronounced halisteresis, scoliosis of the cranial part of the thoracic region of the spinal column and old fractures between the middle and distal thirds of the ulnas, which were apparently united in solid union and perfect position. No definite evidence of tumor invasion of the visualized bones was noted. Blood cultures were negative, but of some significance in view of later disclosures was the presence of occult blood (plus 2) in the stool on both March 20 and 21. Her hospital course was uneventful; she was discharged on April 13 with instructions to adhere to a diet high in calcium and iron and to supplement it with tomato juice and cod liver oil.

She was readmitted Feb. 9, 1939 at the age of 55 years. For four years, or since her discharge in 1935, she had suffered increasingly severe pains in the long bones and the joints, accentuated particularly by motion. She had lost 30 pounds (13.6 Kg.) in this period and was afflicted by occasional attacks of bloating, headache and burning of the eyes following ingestion of the midday meal. The stools had been foamy, gray and foul and frequently contained bits of food eaten a short time before. For three years she had been unable to bear the weight of her body on her feet. Six weeks before, she sprained her right ankle, from which pain did not recede. Examination revealed vital signs practically identical with those recorded on her first admission. She complained of pain in the long bones. Pressure over the joints and long bones elicited dull, grinding pain. The muscles of the legs were moderately atrophic. The skin of the ankles was deeply pigmented over underlying varicosities. The hemoglobin content of the blood was 6.5 Gm. per hundred cubic centimeters, the erythrocyte count 3,040,000 and the total leukocyte count 6,350 per cubic millimeter, with a differential count of 80 per cent polymorphonuclear neutrophils, 16 per cent lymphocytes and 4 per cent eosinophils. The erythrocytes displayed considerable achromia and moderate variation in size and shape. The blood sedimentation rate was 13 per cent in an hour. Chemical examination of the blood gave values as follows: total proteins 5.01 Gm., albumin 2.56 Gm., globulin 2.25 Gm., fibrinogen 0.20 Gm., calcium 9.5 mg., phosphorus 3.9 mg., and nonprotein nitrogen 25 mg. per hundred cubic centimeters. The alkaline phosphatase content of the blood was 12.2 units. Roentgen examination revealed halisteresis in all visualized bones, more prominent than on the previous admission. Also observed was an incomplete fracture through the distal part of the right fibula 6 cm. proximal to the ankle joint and without displacement. Save for a rise of temperature to 100.6 F. on February 12, the course of the patient's illness was uneventful. She was discharged on February 14 with instructions to keep to her diet high in calcium, phosphorus and iron. Not much of her subsequent history could be learned, except that she continued to fail, was largely bedridden and had complaints referable to the skeleton and the gastrointestinal tract. She died at home on Jan. 8, 1940 at 7:30 p. m.

Autopsy (twenty-five and a half hours after death).—The body was that of a white woman aged 56 years, weighing 92 pounds (41.8 Kg.) and measuring 66½ inches (169 cm.) in length. External examination showed edentulism, high grade dorsal hypostasis, great distention and tympanites of the abdomen, a sacral decubital ulcer, a pigmented area in the skin over the distal part of each leg, a complete fracture at the junction of the middle and proximal thirds of the left humerus and a complete fracture of the middle of the left femur.

In the peritoneal cavity, firm adhesions bound the superior surface of the right lobe of the liver to the right leaflet of the diaphragm and the spleen to the lateral peritoneal wall. About 1,500 cc. of puriform opaque yellow fluid was present. A palpable mass in the mesentery of the small intestine was bound to the hepatic flexure of the colon and to coils of the small intestine by firm gray and red adhesions. The diaphragmatic leaflets arched to the level of the fourth ribs bilaterally.

The pleural cavities were entirely obliterated by adhesions. The pericardium, the atrophic thymus and the thyroid gland were not grossly remarkable. The right superior parathyroid gland was located posterior to the right lobe of the thyroid gland and measured 8 by 4 by 3 mm. The left superior gland was in a similar position and measured 12 by 5 by 3 mm. The right inferior parathyroid gland was embedded in the lower pole of the thyroid gland and measured 6 by 3 by 3 mm. The left inferior parathyroid gland was located just inferior to the lower pole of the left lobe of the thyroid gland and measured 12 by 4 by 3 mm. All four parathyroid glands were moderately firm and brown.

The heart weighed 235 Gm. The epicardium, the myocardium, the endocardium and the valves were normal. The coronary arteries were intact save for slight intimal sclerosis in the stem of the left. The aorta also revealed slight intimal sclerosis.

The right lung weighed 535 Gm.; the left, 355 Gm. The surfaces were roughened by adhesions and the lobes were obliterated. The cut section of the lower lobes and of the dependent portions of the upper lobes oozed frothy yellow and sticky dark red fluid, which was also present in the trachea and the bronchi. The pulmonary arteries and veins were intact.

The surface of the 245 Gm., firm, gray-red spleen was partly shaggy at the site of adhesions.

The esophagus was not remarkable. The moderately dilated stomach contained about 100 cc. of thin opaque yellow fluid. The duodenum and coils of jejunum and ileum were distended and contained thin yellow-brown semisolid stuff. In the jejunum, about 165 cm. distal to the pylorus was a large ulcer, 10 cm. long by 7 cm. wide, with firm edges. The ulcer was in close proximity to the mesenteric mass, which measured 13 by 11 by 6.5 cm. and consisted largely of coalescent enlarged lymph nodes. The root of the mesentery adjacent to the mass was dark red and injected. The jejunum entered the jejunal segment bearing the ulcer and emerged from it in such a manner that an L was formed, or a right angle, with the ulcer occupying the junction of the lines of the angle. About 50 cm. of the distal end of the ileum was tightly adherent to the mesenteric mass and some of the tumor had invaded the mesentery and the mucosa of the ileum. Two red-yellow, friable masses of neoplastic tissue directly adjacent to each other and measuring 3.5 by 3 cm. and 2 by 2 cm. in area were ulcerated through

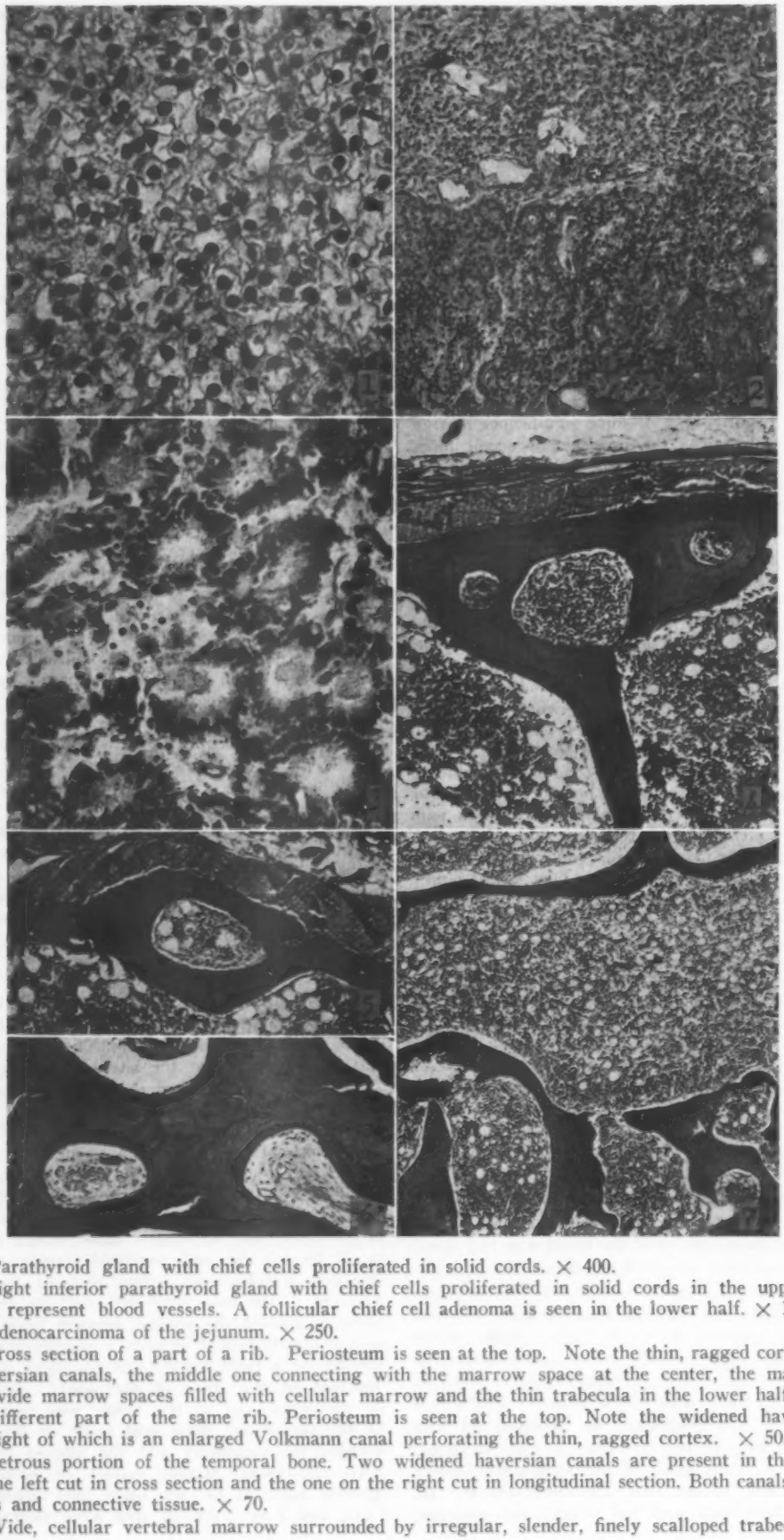

Fig. 1.—Parathyroid gland with chief cells proliferated in solid cords. × 400.

Fig. 2.—Right inferior parathyroid gland with chief cells proliferated in solid cords in the upper half; the clear spaces represent blood vessels. A follicular chief cell adenoma is seen in the lower half. × 125.

Fig. 3.—Adenocarcinoma of the jejunum. × 250.

Fig. 4.—Cross section of a part of a rib. Periosteum is seen at the top. Note the thin, ragged cortex, the three widened haversian canals, the middle one connecting with the marrow space at the center, the marrow in the canals, the wide marrow spaces filled with cellular marrow and the thin trabecula in the lower half. × 50.

Fig. 5.—Different part of the same rib. Periosteum is seen at the top. Note the widened haversian canal just to the right of which is an enlarged Volkmann canal perforating the thin, ragged cortex. × 50.

Fig. 6.—Petrous portion of the temporal bone. Two widened haversian canals are present in the lower half, the one on the left cut in cross section and the one on the right cut in longitudinal section. Both canals contain new blood vessels and connective tissue. × 70.

Fig. 7.—Wide, cellular vertebral marrow surrounded by irregular, slender, finely scalloped trabeculae. × 25.

the mucosa of the colon at a point in the hepatic flexure 30 cm. distal to the appendix. About 60 cm. proximal to the anus in the mucosa of the colon was a dark red area measuring 14.5 by 4.5 cm. The appendix was 4 cm. long, 6 mm. in diameter and intact.

The liver weighed 1,675 Gm., and the capsule was intact. The parenchyma was fairly firm and light brown-yellow. The lobules were indistinct. The biliary ducts and the hepatic blood vessels were well preserved. The coats of the gallbladder were stretched by about 100 cc. of dark amber viscid fluid. The pancreatic parenchyma, ducts and blood vessels were intact. The adrenal glands were grossly normal.

Each kidney weighed 135 Gm. The capsules stripped easily, revealing slightly granular, focally pitted surfaces. The cortices were 3 to 5 mm. thick, and the striations were focally indistinct. The renal medullae and pelves, the ureters and the urinary bladder were well preserved.

The uterus, the fallopian tubes and the left ovary were intact. The right ovary measured 3.5 by 2 by 1.5 cm. and contained a 1.5 cm simple cyst.

Two stony hard mediastinal lymph nodes were 1.5 and 2.5 cm. in diameter. The mesenteric nodes not included in the mesenteric mass described were discrete and ranged up to 15 mm. in diameter.

The marrow in the ribs and the vertebrae was pink-gray and mottled with white patches.

The brain weighed 1,440 Gm. and was grossly normal as shown by transverse serial sections through the cerebral hemispheres, the pons, the medulla and the cerebellum. The pituitary gland was grossly intact.

The skull, the ribs and the vertebrae cut easily with a rib knife to reveal very thin, easily broken bony trabeculae. The cranial part of the thoracic portion of the spinal column showed a slight bowing to the right.

Microscopic Examination.—The tissues were fixed in Zenker's fluid, embedded in paraffin, cut at 6 microns' thickness and stained by the hematoxylin-eosin method. The significant changes aside from those in the parathyroid glands, the jejunum and the skeleton (ribs, vertebrae and skull) were as follows: incomplete involution of the thymic parenchyma, physiologic atrophy of the thyroid gland, fibrous pleuritis, pulmonary hemorrhage and edema, chronic suppurative peritonitis, severe fatty metamorphosis of the liver, moderate arteriosclerosis of the kidneys, simple cyst of the right ovary, healed tubercles in the mediastinal lymph nodes and ectopic bone marrow in the arachnoid adjacent to the base of the pituitary gland. The heart, the spleen, the pancreas, the adrenal glands, the uterus, the brain and the pituitary gland were histologically normal.

Parathyroid Glands: All four glands showed hyperplasia of the chief cells, which were arranged mainly in solid cords (fig. 1) but also in follicular fashion in several areas. Fat cells were rare, and pale oxyphilic cells were occasional. A few simple epithelial cysts in one gland contained homogeneous acidophilic material. The right inferior parathyroid gland also showed an adenoma (fig. 2) 3 mm. in its greatest diameter when measured in the histologic section. This adenoma was composed of chief cells in definite glandular arrangement with basal orientation of the nuclei of the cells and acidophilic secretion in the lumens of the follicles. This adenoma was clearly demarcated from the adjacent hyperplastic parathyroid tissue.

Jejunum and Adjacent Tissues: Anaplastic columnar epithelial cells in an irregular, atypical glandular pattern (fig. 3) arose from the mucosa of the jejunum and invaded the entire wall. Many necrotic areas marked the neoplastic tissue. All coats of the colon at the sites described grossly were invaded by neoplasm like that in the jejunum, and the mucosa of the colon was ulcerated. Several mesenteric lymph nodes were replaced by masses of adenocarcinoma like that seen in the jejunum.

Bones: The sections of bone were lightly decalcified in 5 per cent nitric acid and stained by the hematoxylin-eosin method after being embedded in paraffin and cut at 6 microns. A rib revealed great thinning of the cortical bone as well as of the trabeculae of the medullary bone, with raggedness and fine scalloping of the edges and concomitant decrease of osteocytes, but without osteoclastic reaction. The canals of Volkmann and the haversian canals were increased in width, some of the latter to a striking degree (fig. 4). In many enlarged haversian canals were fine new connective tissue and red marrow (fig. 5). The marrow spaces were tremendously widened. Sections of squeezed out rib marrow were stained by Giemsa's method, and smears of rib marrow were stained by Wright's method. The marrow cell/fat cell ratio averaged 80/20. The myeloid/erythroid ratio was greatly increased by increase of the cells of the neutrophilic granulocyte line, which showed a shift to the left as evinced by the many metamyelocyte, myelocyte and promyelocyte forms. Megakaryocytes were plentiful, and occasional small lymphatic nodules were seen. Except for the concomitant decrease in the nucleated forms of the erythrocyte line represented by scattered mature normoblasts, no disturbance of erythrocytopoiesis was observed. The costochondral junction of one rib did not reveal significant changes, nor did the costal cartilage. With the same technic, sections of vertebrae (fig. 7) and of the petrous portions of the temporal bones (fig. 6) showed bony changes like those described for the ribs. The vertebral marrow was similar to the rib marrow, but the marrow of the fragments of temporal bone was largely fatty. The Hansen and Bock method of staining with hematoxylin and counterstaining with eosin was also employed in the study of the vertebrae. This stain indicated a pronounced dearth of lime salts as demonstrated by the pale polychromatophilic tingling of the bony trabeculae.

The final anatomic diagnosis was: adenocarcinoma of the jejunum with extension to the peritoneum, the ileum and the colon, chronic suppurative peritonitis, and metastases to the mesenteric lymph nodes; pulmonary edema and hemorrhage; hyperplasia of the parathyroid glands; follicular adenoma of the right inferior parathyroid gland; generalized osteoporosis; fractures of the left humerus and the left femur; severe fatty metamorphosis of the liver; moderate arteriosclerosis of the kidneys; myeloid hyperplasia of the marrow; ectopic bone marrow in the arachnoid at the base of the pituitary gland; incomplete involution of the thymus; healed tubercles of the mediastinal lymph nodes; simple cyst of the right ovary; bilateral obliterative fibrous pleuritis; sacral decubital ulcer.

COMMENT

According to the history, the patient had diarrhea for eighteen years, at first intermittently and finally almost continuously. Under this condition of chronic intestinal hypermotility produced by the adenocarcinoma of the jejunum, calcium and phosphorus were probably badly absorbed from the intestine. This resulted in

lowered levels in the blood, where the concentration of these minerals is maintained at all costs, and thus a chronic strain was placed on the parathyroid glands. Under their influence the skeleton was forced to yield tertiary calcium phosphate to replenish the need for calcium and phosphate in the blood. Since the functional renal parenchyma and the colon were largely intact, excretion of these ions was relatively normal. The relative soundness of the kidneys also served to explain the absence of metastatic calcification, as did the relatively low blood levels of calcium and phosphorus observed on two occasions. The single value of alkaline phosphatase two to three times normal indicated definite destruction of bone, so well demonstrated in the skeletal roentgenograms. Malabsorption of vitamin D was probably somewhat compensated by the large amount, about 70 per cent, of solar radiation available yearly in Colorado.

The parathyroid glands in this patient were definitely hyperplastic. The volumetric measurements were as follows: right superior, 96 cu. mm.; left superior, 180 cu. mm.; right inferior, 54 cu. mm., and left inferior, 144 cu. mm., making a total volume of 474 cu. mm. Except for the right inferior gland, which contained the follicular chief cell adenoma, the parathyroid glands were much larger in volume than the largest normal volume possible, namely, 56 cu. mm., given as the outside limit of normal by Castleman and Mallory.[3] The histologic changes in the parathyroid glands of this patient agreed with those described for hyperplasia of these glands in chronic renal insufficiency, particularly in chronic glomerulonephritis.[4]

The severe fatty metamorphosis of the liver might have been caused by malabsorption of carbohydrates, of proteins and of vitamins, especially of the B complex and specifically nicotinic acid, as well as of substances like choline and methionine.[7] The chronically deranged liver cell was less able to manufacture albumin, low on two occasions in the blood of this patient, thus decreasing the total bound calcium in the blood. The fall of bound calcium depleted a reservoir for the replenishment of ionic calcium. Not inconceivable was the shifting of part of the remaining bound calcium to the ionic state so that excretion of this ion through the kidneys and colon was facilitated. This placed a strain on the parathyroid glands by the necessity of calling forth tertiary calcium phosphate from the bones in an attempt to furnish calcium ions to maintain a proper balance between bound and ionic calcium.[8] The chronically upset liver cell was less efficient in phosphorylation and phospholipid turnover,[7] leaving a probably greater quantity of phosphate in the ionic state and thus allowing it to be excreted with greater ease by the relatively little damaged kidneys and colon, placing added demand on the parathyroid glands. Of interest in connection with chronic damage of the liver in the production of hyperplasia of the parathyroid glands was the case of a man 64 years old, who had been a heavy consumer of ethyl alcohol in the form of 1 quart (about 1,000 cc.) of whisky a day for at least seven years. Autopsy revealed severe fatty metamorphosis, recent central necrosis and early periportal cirrhosis of the liver. His inferior parathyroid glands showed chief cell hyperplasia, although not to the degree in the case now reported.

The intestinal hypermotility and the damage of the liver could have explained the failure of both absorption and storage of the erythrocyte-maturing factor and iron, which were partly responsible for the anemia, the latter being partly due to intestinal loss of blood from the adenocarcinoma of the jejunum as indicated by the finding of occult blood in the stool on two occasions during the first hospitalization of the patient. The increased granulocytopoiesis in the marrow was demanded by the chronic suppurative peritonitis and resulted in crowding out of erythrocytopoiesis to add another factor in the production of the anemia. The marrow did not show any evidence of a specific lack of the erythrocyte-maturing factor.

The unusual fractures of the ulnas and of the right fibula, and possibly of the left humerus, represented a strong clinical indication that some diffuse skeletal disease was the underlying condition rather than trauma alone. Although the symptoms of the patient at the time of injection of the gold salt were undoubtedly due to the heavy metal, it is hardly justifiable to attribute to the gold the changes found at autopsy eight years later.

SUMMARY

In a case of adenocarcinoma of the jejunum, the associated hyperplasia of the parathyroid glands and the generalized osteoporosis seemed to be on the basis of chronic intestinal hypermotility. In addition, it has been suggested that a chronically damaged liver may play a role in the causation of hyperplasia of the parathyroid glands.

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CHRONIC JEJUNITIS

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Nonspecific granulomatous inflammation of the terminal part of the ileum was first described as a clinical entity in 1932 by Crohn, Ginzburg and Oppenheimer.[1] Subsequent to their original publication the morbid process which they recognized has been found in the jejunum by Harris, Bell and Brunn,[2] Crohn,[3] Brown and Donald [4] and others. For this reason and because the lesion has also been found in the colon, the condition originally designated as "regional ileitis" has been termed "regional enteritis."

The pathologic picture observed by the various authors has been consistent. Holloway [5] divided the course of the inflammatory process into three stages: acute, subacute and chronic. He described the chronic form as being characterized grossly by thickening, rigidity and tumor formation in the involved intestine, with extensive inflammatory reaction in the lymph nodes and the adjacent mesentery. Microscopically, the mucosa shows ulceration of varying degree and depth, with thickening of the walls. There is also stenosis due to fibrosis, with an infiltration of inflammatory cells which may vary with the severity of the reaction. Granulomas in the wall of the bowel with tubercle formation and foreign body giant cells have been described by Meyer and Rosi,[6] Clark and Dixon [7] and Sirin.[8] Although the tubercles resemble those of tuberculosis in some measure, the giant cells seen have not usually been of the Langhans type, and tubercle bacilli have never been demonstrated. Clark and Dixon [7] discovered foreign bodies within the giant cells, which they interpreted as being remnants of food that lodged in the ulcerated mucosa.

Recently a patient was admitted to the regional hospital at a camp in Mississippi because of an intestinal obstruction. At operation a stenosing granulomatous lesion involving the proximal portion of the jejunum was resected. Although nonspecific inflammations of the jejunum have been observed before, there are relatively few cases of jejunitis recorded. For this reason, and because of the unusual pathologic changes observed, the case is considered to be of sufficient interest to report in the literature.

REPORT OF A CASE

The patient was a 25 year old white soldier with over three years' service in the Army. He was admitted to the hospital, March 27, 1944, complaining of abdominal pain and vomiting. He stated that symptoms referable to the gastrointestinal tract first developed one and one-half years prior to the present admission while he was stationed at a camp in Massachusetts. At that time he had occasional epigastric pain and postprandial vomiting. A series of gastrointestional roentgenograms made in Massachusetts was reported as showing nothing abnormal. His symptoms became progressively worse until at the time of admission he had fairly constant sharp periumbilic pain, which was aggravated by eating any type of food. The pain was associated with gurgling noises, gas, belching and almost regularly nausea and vomiting. About one month prior to admission he noticed an "egg-shaped" swelling in each lower quadrant of the abdomen, chiefly on the left. The swellings appeared and disappeared suddenly at frequent intervals. Except for occasional constipation, his bowel movements were essentially normal until one month prior to admission, when his stools (one a day) became soft, sticky and liquid. He had never noticed any pus or blood. He stated that he had lost about 30 pounds (13.5 Kg.) since the onset of his illness. His habits had always been moderate. His family history was noncontributory. He had been born and reared in Massachusetts, and for about two years before enlistment in the Army he had worked in the CCC and with the Forestry Service in New Hampshire. He had had the usual diseases of childhood but never any serious illness or operation.

The patient's temperature was 98.6 F. The blood pressure was 118 systolic and 65 diastolic. The pulse rate was 72. The patient was an emaciated white man who appeared chronically ill. His skin was sallow. The abdomen was scaphoid and soft. There was moderate tenderness along the sigmoid and the descending colon. During the examination a large pear-shaped mass suddenly appeared in the left lower quadrant, with its apex pointing cephalad. It was soft and tympanitic, and on slight pressure disappeared with a loud gurgling noise. Rectal examination revealed marked tenderness with a suggestion of fulness anteriorly. Proctoscopic examination showed nothing unusual. The physical examination yielded no other evidence of abnormality.

The urine on admission was normal. The hemoglobin content was 80 per cent (Tallqvist); the red blood cell count was 4,250,000; the white blood cell count was 5,550, with segmented cells 57 per cent, stab cells 3 per

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cent and lymphocytes 40 per cent. A subsequent blood count showed the hemoglobin content 80 per cent (Tallqvist), the red blood cell count 4,000,000 and the white cell count 5,450 with segmented cells 56 per cent, stab cells 4 per cent and lymphocytes 40 per cent.

A series of gastrointestinal roentgenograms showed the esophagus, the stomach and the duodenal cap to be normally outlined. After leaving the cap the barium sulfate outlined a moderately dilated remaining duodenum and showed about 2 feet (61 cm.) of jejunum to end in the region of the pelvic inlet in an area of almost complete obstruction. About 5 inches (12.5 cm.) proximal to the obstruction the bowel was constricted. A small amount of barium sulfate passed through to fill a somewhat ragged and moth-eaten segment of small bowel.

Roentgenograms of the colon obtained after barium sulfate enemas on two different occasions revealed no lesion.

April 6, 1944, a reddened, thickened, dilated portion of the proximal part of the jejunum together with its attached mesentery was excised. Exploration of the remainder of the peritoneal cavity at the time of operation did not reveal any inflammatory reaction in either the ileum or the colon. The only enlarged mesenteric nodes encountered were those in the region of the inflamed jejunum. The continuity of the bowel was reestablished with a side to side anastomosis of the cut ends.

The patient made a rather uneventful recovery. Three weeks later he had regained 14 pounds (6.5 Kg.). During his convalescence, however, he continued to have four to six stools a day with considerable gas. Finally the frequency of bowel movements subsided so that by the time of discharge, June 20, 1944, he was once more comfortable. A postoperative series of gastrointestinal roentgenograms showed no further evidence of obstruction or other pathologic process.

Pathologic Observations.—Grossly, the specimen consisted of a portion of the jejunum 55 cm. long, with its attached mesentery. The proximal 27 cm. was tremendously dilated, measuring about 8 cm. in diameter. On section through this portion, the wall showed some thickening, and the rugae of the mucosa were flattened to a great degree. The distal 28 cm. was firm, hard and hoselike. The serosal surface contained many small hemorrhagic areas and had a pebbly gelatinous appearance. On section the thickened wall was firm when cut and was a translucent grayish color. In some areas the jejunal wall was 1 cm. thick and rigid, constricting the lumen to about 3 mm. The mucosa was ulcerated in several areas, and the lumen contained a pearl gray mucoid material. The mesentery was markedly thickened, and its vessels were congested. The lymph nodes were enlarged and rubbery. On section, the nodes had a pinkish gray homogeneous color, and the parenchyma bulged from the capsule.

Microscopically, the thickened portion of bowel showed marked edema, most evident beneath the serosa, where there were numerous widely dilated blood vessels, as well as an infiltration of scattered lymphocytes and plasma cells. The muscularis appeared to be intact, although it had been interrupted in several places by areas of fibrous tissue proliferation. In the submucosa there were considerable edema, areas of fibrosis and a dense infiltration of round cells. In some areas abscess formations and sinus tracts traversed the submucosa. They were filled with purulent material, and their walls were formed by an edematous inflammatory tissue containing a dense polymorphonuclear leukocyte infiltration. In some of the abscesses colonies of bacilli were seen. The mucosa was intact and hyperplastic in some places, but areas of ulceration were present.

Tubercles could be found throughout all portions of the thickened intestine. They consisted of a large central giant cell, usually enclosing a deeply basophilic foreign body and surrounded by a zone of epithelioid cells and lymphocytes. Most of these were found between the circular and the longitudinal muscle layer (fig. 1). Some of the giant cells and their surrounding epithelioid cells appeared to be lying in, and attached to, the walls of cystic spaces, which contained round and segmented cells and deposits of albuminous material. Lymphocytic infiltration surrounded these spaces (fig. 2).

In the submucosa several foreign bodies lay free, surrounded only by round cells, without any tubercle formation (fig. 3). These appeared to be more recent invaders than those found lying between the muscular layers. Numerous well defined aggregates of lymphocytes were found in the submucosa and the subserosa. Serial sections demonstrated that these were the peripheral zones of tubercles which contained foreign bodies and giant cells in their central portions.

The lymph sinuses of the mesenteric nodes were tremendously dilated, and there was marked proliferation of reticulum cells. In some areas the germinal follicles appeared to be replaced by depositions of amyloid (fig. 4). No tubercles could be demonstrated in any of the regional lymph nodes.

COMMENT

Because of the striking number of foreign bodies with their surrounding tubercle formations in the sections, and because some of the foreign bodies resembled round worms (fig. 3), the possibility of a parasitic infection suggested itself. The parasite commonly found in the upper part of the jejunum, one which invades the bowel wall, is Strongyloides stercoralis.

The female worm tunnels beneath the mucosa where she lays her eggs. These then develop into rhabditiform larvae, reenter the bowel and are passed with the feces, in which the intermediate cycle of the parasite takes place with the development of the filariform larval stage.[9] Usually neither the adult worms nor the larvae are found beneath the muscularis mucosae. Faust and deGroat [10] have shown, however, that autoreinfection with Strongyloides is possible, and they have described a case in which the entire thickness of the ileal wall of a 12 year old boy had been invaded by the filariform larvae, giving rise to inflammation and mucosal ulceration. Both these authors and Hinman [11] mentioned the possibility that the parasite liberates a lytic substance causing mechanical damage of the bowel wall and the relative absence of cellular reaction other than eosinophilia. Hinman, however, stated that occasionally the worms become encapsulated by tissue reaction and that the eosinophilia is variable, at times being within

9. Strong, R. P.: Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases, ed. 6, Philadelphia, The Blakiston Company, 1942, vol. 2, pp. 1278-1287.

10. Faust, E. C., and deGroat, A: Am. J. Trop. Med. **20**:359, 1940.

11. Hinman, E. H.: Rev. Gastroenterol. **5**:24, 1938.

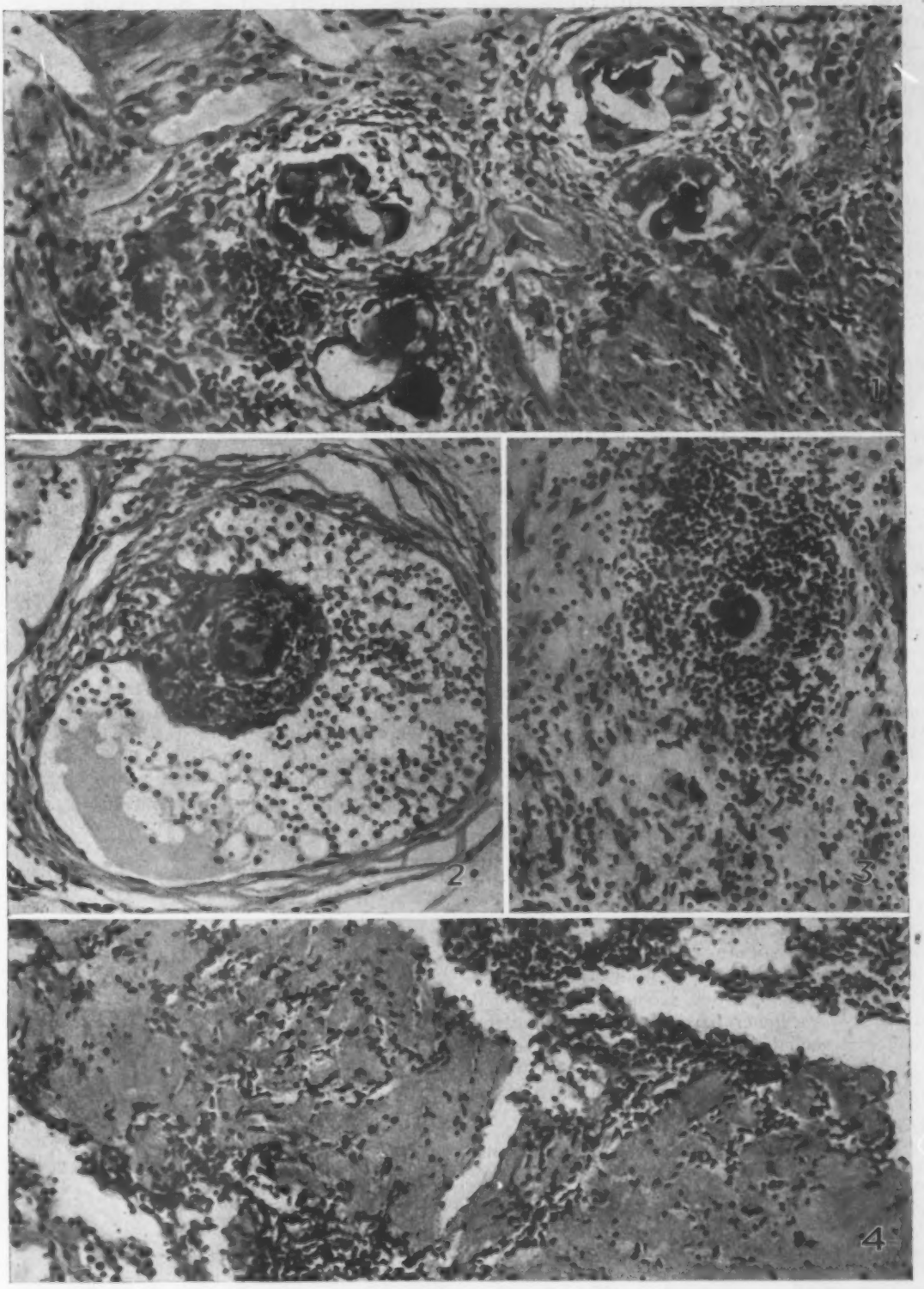

Fig. 1.—Foreign body granulomas lying between muscle layers. (United States Army Medical Museum, negative 83821.)

Fig. 2.—Granuloma with surrounding cystic space. (United States Army Medical Museum, negative 83819.)

Fig. 3.—Foreign body lying in submucosa, resembling a larva of Strongyloides stercoralis. (United States Army Medical Museum, negative 83822.)

Fig. 4.—Amyloid infiltration into a mesenteric lymph node. (United States Army Medical Museum, negative 83820.)

normal limits. Ophuls [12] reported the death and autopsy of a patient who, although he had a massive infection of the region of the pylorus, never had over 3 per cent eosinophils in the peripheral blood.

From the evidence offered by these authors it is conceivably possible to have an infection of the bowel wall in all its coats with the larvae of Strongyloides without eosinophilic reaction and with encapsulation of the parasites by tissue reaction. Moss [13] and Hood, after reviewing the sections in the present case, agreed that the foreign bodies seen could possibly be larvae of S. stercoralis.

Subsequent to operation 22 examinations of the stools of the patient were made in an attempt to find intestinal parasites. All yielded negative results.

Microscopic sections and wet tissues were forwarded to the Army Institute of Pathology and their report is as follows:

> A review of the sections submitted as well as of additional sections prepared from the gross material has led to the impression that we are dealing in this case with a foreign body reaction to some unknown organism. We have not been able to confirm your impression that larvae of Strongyloides stercoralis are present.
>
> In the differential diagnosis of lesions of this type one has to consider not only foreign body reaction but also regional or segmental enteritis, venereal granuloma and sarcoidosis. The concretions present in the giant cells bear a not too distant resemblance to the Schaumann bodies of sarcoidosis.
>
> We do not feel that it is possible at the present time to account definitively for the origin or the nature of the lesion present in this interesting specimen.

As suggested in this report, other diseases associated with granulomatous lesions had been considered but were discarded as not being the probable cause of the pathologic changes for various reasons.

Studies made by Schaumann,[14] Snapper and Pompen [15] and numerous European investigators, in addition to Longcope,[16] Rubin and Pinner,[17] and others in this country, have well established sarcoidosis as a generalized disease affecting the reticuloendothelial system. In a careful search of the literature no cases were found in which the small intestine was the site of sarcoidosis without involvement of other organs. Clinically and roentgenographically there was no sign of any disease elsewhere in the body of our patient.

Although the deeply basophilic foreign bodies seen in the tubercles resembled the concretions found in the tubercles in sarcoidosis, the granulomatous reaction did not suggest sarcoidosis. Nowhere was the epithelioid cell proliferation as extensive as it is in sarcoidosis. Lesions represented by that illustrated in figure 3 gave the impression that the foreign bodies preceded the tubercle formation and did not result from degeneration of the central portion of the giant cells as in sarcoidosis. Also, the reaction seen was inflammatory rather than proliferative.

Since sarcoidosis primarily affects the reticuloendothelial system, one might expect to find evidence of it in the regional mesenteric lymph nodes if one were dealing with that disease. As previously described, the mesenteric nodes showed only secondary inflammatory hyperplasia and some amyloid infiltration.

Venereal granuloma attacks the rectum and the lower part of the colon as a complication of the primary infection, the latter extending to those structures through the pelvic lymphatics. Occasionally in the male the rectum is the site of the primary infection. However, involvement of the upper portion of the intestinal tract has never been described. The presence of foreign bodies such as one sees in the present case has not been demonstrated in the granulomatous lesions of venereal lymphogranuloma, and in the absence of a history of inguinal lymphadenopathy and with no evidence of disease in the rectum, the possibility of this being a manifestation of venereal granuloma is unlikely.

It is my impression that in this case there was regional enteritis located high in the jejunum without characteristic "skip" areas and with a prominent foreign body invasion. Because the foreign bodies were so numerous and located principally between the muscle coats, and because some of them resembled parasitic larvae, the question was raised whether or not there might be some other factor involved in the process. If the foreign bodies seen were larvae of S. stercoralis, it would be difficult to say whether the inflammatory process was caused by the infection or whether it was an independent lesion, either preceding or succeeding the parasitic invasion.

SUMMARY

In a case of chronic jejunitis numerous tubercle-like granulomas were seen in association with a considerable amount of foreign material. None of the etiologic factors which might have been involved was established as the cause.

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13. Moss, E. S.: Personal communication to the author.
14. Schaumann, J.: Brit. J. Dermat. **48**:399, 1936.
15. Snapper, I., and Pompen, A. W. M.: Pseudo-Tuberculosis in Man: I. Sarcoid of Besnier-Boeck, Haarlem, de Erven F. Bohn, 1938; reviewed, J. A. M. A. **112**:175, 1939.
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General Reviews

MORPHOLOGIC ASPECTS AND GENESIS OF DISORDERS OF THE ADENOHYPOPHYSIS

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The disorders of the adenohypophysis can be classified as those caused by increased activity of the gland and those due to partial or total deficiency of the gland. These conditions are denoted by the terms "hyperpituitarism" and "hypopituitarism" or "apituitarism." The former is represented by acromegaly and hypophysial gigantism, whereas the latter comprises hypophysial dwarfism, Fröhlich's syndrome and hypophysial cachexia. Brugsch's acromicria or osteogenital dystrophy might be a form of hypopituitarism, but so far there seem to be no reliable postmortem observations indicating this. In Cushing's syndrome, called by Cushing himself "pituitary basophilism," the primary hypophysial origin of the disorder and its hyperpituitaristic nature appear, for reasons to be discussed later, debatable; this syndrome will be taken up separately at the end of the paper.

I

ACROMEGALY (MARIE'S SYNDROME)

Acromegaly is the sequel of eosinophilic hyperpituitarism or, in other words, the result of hyperfunction of the eosinophilic cells of the hypophysis, known as the producers of the growth hormone. The overproduction of this hormone causes enlargement of the prominent parts of the body, such as the nose, the lips, the ears, the lower jaw, the hands and the feet, and there are also hyperplastic changes of the skeleton, macroglossia and splenomegaly. Marked hairiness, with the masculine type of hair in females, cessation of the sexual functions, glycosuria, diabetes mellitus, certain visual disturbances, especially bitemporal hemianopsia, symptoms of increased brain pressure and changes of the blood picture, such as mononucleosis and eosinophilia, complete the clinical picture. The disease usually starts between 20 and 30 years of age. Not unusual is a beginning in puberty. Children rarely present acromegaly, and Salle's observation of the condition in a tiny infant must be called unique.[1] (See also Behrens and Barr.[2])

Acromegaly is a disease of long duration, lasting even twenty, twenty-five and more years. The period of active growth, however, is relatively short. The two sexes are equally involved by the disease (Davidoff [3]).

Fugitive acromegaly (so-called by Bailey and Cushing [4]) is a mild form of the disease showing one or more of the following signs or symptoms: enlargement of the hands and the feet, coarsening of the features, hypertrichosis, exaggerated libido, excessive height, increased perspiration, squaring and tufting of the phalanges, persistent lactation, glycosuria and slightly elevated basal metabolism. Recognizable traces of hyperpituitarism are apparently present from the onset.

The morphologic substratum of the hyperpituitarism causing acromegaly is, as mentioned, the eosinophilic adenoma, cancerous or noncancerous, originating as a rule in the anterior lobe itself; in extremely rare cases, however, it may arise in the region of the former craniopharyngial canal. In a few instances diffuse or nodular hyperplasia of the eosinophilic cells has been observed, particularly in the rare cases of infantile acromegaly (Salle [1]; Lewis [5]).

The eosinophilic adenoma varies in size and the symptoms are not always in conformity with the size. Thus one can see a small tumor of this type accompanied by the fully developed classic picture of acromegaly, whereas in another case, despite a large tumor, the acromegalic symptoms are only slightly evident. The size of the eosinophilic adenoma is not so responsible for the severity of the acromegalic symptoms as the degree of the functional differentiation of the cells

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1. Salle, cited by Kraus.[15]
2. Behrens, L. H., and Barr, D. P.: Endocrinology **16**:120, 1932.
3. Davidoff, L. M.: Endocrinology **10**:453, 1926.
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5. Lewis, D.: Bull. Johns Hopkins Hosp. **16**:151, 1905.

of the tumor. The more the cells simulate normal eosinophils, the more effective is the adenoma as an endocrine factor.

There are many statements, particularly in the older literature, about cases of acromegaly without eosinophilic tumor. This is partially explained by the fact that the eosinophilic cells in old adenoma may lose, at least to a great extent, their specific granules because of exhaustion. This has been reported recently by Spark and Biller [6] in a case of acromegaly of twenty-one years' duration. Here the period of active growth lasted for a relatively short time, and at autopsy the main bulk of the cells of the adenoma showed complete loss of the specific granulation. Faulty staining of the sections is responsible for some other cases of acromegaly "without" eosinophilic adenoma. The demonstration of the specific granulation in the eosinophilic adenoma may be difficult if the tumor cells are not differentiated enough. Often the granules can be demonstrated reliably only by certain methods, such as the Mallory-Heidenhain stain, with azocarmine G, or the chromium-hematoxylin stain described by Kraus.[7] Likewise, confusion of acromegaly with gigantism, hypertrophic pneumonic osteoarthropathy, syringomyelia, megalocheiria and megalopodia is accountable for the assertion that acromegaly need not be associated with eosinophilic adenoma. In rare instances the eosinophilic adenoma may be hidden within the sphenoid bone while the hypophysis and the sella turcica appear normal (Erdheim [8]). Theoretically, the eosinophilic adenoma causing acromegaly can also originate in the pharyngeal hypophysis, but so far no case of acromegaly with the eosinophilic adenoma in this region seems to have been observed.

The assertion that true acromegaly may be caused by any other condition than eosinophilic hyperpituitarism is incorrect. Therefore, the statement by Werner [9] that syphilis has produced acromegaly must be questioned.

In addition to the hypophysis, most of the other endocrine organs are involved. The pineal gland frequently appears enlarged; the thyroid gland often (according to Davis,[10] in 50 per cent of the cases) shows nodular or diffuse goiter with colloid degeneration, atrophy of the parenchyma and increase of fibrous tissue, sometimes complicated by myxedema. The parathyroid glands may be hyperplastic, and also the thymus, which may show an increase of cortex, medulla and Hassall's corpuscles. The pancreas, as a rule, is enlarged because of proliferated interstitial tissue. There may be hydropic degeneration, atrophy and depletion of Langerhans' islets, as well as fibrosis and hyalinization. These lesions can be found even in cases without manifest disturbance of the carbohydrate metabolism (Kraus and Reisinger [11]). In the adrenal glands the hyperplasia concerns the cortex as well as the medulla. The combined weights of these glands may be as high as 25 Gm. and possibly higher. According to Cushing and Davidoff,[12] enlargement of the adrenal glands is more frequent than goiter. In rare instances tumors may be found in several endocrine glands aside from the eosinophilic adenoma in the hypophysis, for instance in the pineal, the thyroid, the parathyroid and the adrenal glands and the pancreas. The testes, particularly when the disorder is advanced, show atrophy of the seminiferous canaliculi and depletion of Leydig's interstitial cells, the disappearance of which in the early stages of testicular atrophy is characteristic of the hypophysiodiencephalic disorganization. Cystic degeneration and atrophy of primordial and graafian follicles are typical findings in the ovaries. In other cases, however, many years after acromegaly has started, menstruation still can be present, just as in males spermatogenesis may be perfect despite many years of acromegaly. Hypoplasia of the genitalia has been observed in cases of juvenile acromegaly.

Hirsch,[13] who operated on 190 patients with tumor of the hypophysis, stated that among 87 women there were 77 less than 46 years of age and therefore able to menstruate, but because of the hypophysial tumor 87 per cent of them had amenorrhea. Of these 77 women, 14 had acromegaly, and 43 per cent of of these acromegalic women were menstruating, while only 6.3 per cent of the 63 patients with hypophysial tumor without acromegaly still had their menses. In other words, among women with hypophysial tumor without acromegaly 93.5 per cent were amenorrheal, while among acromegalic women only 57 per cent showed genital disturbances.

Important for the sexual function is the extent to which the hypophysial parenchyma is destroyed and the interbrain injured by the hypophysial tumor. Since in many cases of acromegaly the eosinophilic adenoma is not too large,

6. Spark, C., and Biller, S.: Arch. Path. **35**:93, 1943.
7. Kraus, E. J.: Frankfurt. Ztschr. f. Path. **10**:161, 1912.
8. Erdheim, J.: Beitr. z. path. Anat. u. z. allg. Path. **46**:233, 1909.
9. Werner, A. A.: J. South. M. A. **24**:953, 1931.
10. Davis, A. C.: J. Clin. Endocrinol. **1**:445, 1941.
11. Kraus, E. J., and Reisinger, A.: Frankfurt. Ztschr. f. Path. **30**:68, 1924.
12. Cushing, H., and Davidoff, L. M.: Arch. Int. Med. **39**:673, 1927.
13. Hirsch, O.: Presse méd. **34**:578, 1926.

there is often more surviving hypophysial parenchyma and lesser injury to the interbrain than is found in cases of chromophobe adenoma or craniopharyngioma. This may be the reason why genital disorders are less frequent in patients with acromegaly than in patients with other types of hypophysial tumor. The slow growth of eosinophilic adenoma in many cases might explain why sexual insufficiency often is not found in the first years of the disease. Even signs of sexual hyperactivity can be frequently observed in patients, especially males, in whom the disease is in its early stages.

The enlargement of the nose, the ears, the lips—particularly the lower lip—and the thickening of the eyelids are due mainly to proliferated fibrous tissue, but the epidermis also participates in the thickening of these structures. In the nose and the larynx the cartilage is hyperplastic. The low-pitched rough voice of the acromegalic patient results at least partly from the thickening of the pharyngeal and laryngeal mucosa. The protrusion of the eyeballs observed in some patients is caused by an increase of retrobulbar tissue. Splanchnomegaly mainly results from the proliferation of interstitial tissue. In addition to the endocrine organs mentioned, the organs usually enlarged are the tongue, the larynx and the trachea, the heart, the liver, the kidneys, the pancreas and the gastrointestinal canal. I have recorded excessive weights for the organs of a male acromegalic patient 176 cm. in length, the heart weighing 950 Gm., the liver 3,770 Gm., the spleen 650 Gm., the thyroid gland and the pancreas 150 Gm. each and both adrenal glands 25 Gm. Hyperplasia of the lymphatic tissue is frequent. The brain and the spinal cord are in some cases enlarged. Destructive changes in the optic nerves are common. In the nerve cells of the supraoptic and paraventricular nuclei, vacuoles and degenerative atrophy have been seen, the latter also being found in the corpora mammillaria. The sympathetic and spinal nerves often show proliferation of fibrous tissue. In later stages of the disease, atrophy of the musculature with following fibrosis may be seen. Atherosclerosis of the arteries is frequent, and so is thickening of the skin, which often presents fibromas, warts, comedos and acne-like pustules.

The skeleton shows thickening of the skull, with the normally protuberant parts, such as the supraorbital margin, the zygomatic bone, the occipital protuberance and the osseous gyri, strikingly prominent. The turbinate bones of the nose participate in the enlargement. The pneumatic nasal cavities are widened, and the lower jaw is enlarged, causing prognathism, with the lower teeth overlapping the upper teeth sometimes as much as 2 cm. Separation of teeth and thickening of the collar bones, the ribs and the epiphyses of the long bones are frequent. Osteophytic proliferation on the surfaces of the bones, exostoses at the ends of the bones close to the joints and thickening of the muscle insertions, of the manubrium sterni and of the phalanges of the fingers and the toes are typical changes. The hands show two types of enlargement, one in which they are enlarged in length (*type en long*) and one in which they are enlarged in width (*type en large*). Kyphosis in the lower cervical and upper thoracic portions of the spinal column and lordosis of the lumbar portion are frequently observed in acromegalic persons. In later stages of the disease, osteoporotic changes appear, associated with atrophy of the musculature, especially of the arms and the legs. Thickening of the bones can be found together with porosis. The lack of osteoblasts, osteoclasts and lacunar resorption of bone indicates slow transformation of the internal bone structure. The spongy bone increases at the expense of the compact bone, and the latter consists of concentrically arranged lamellas, surrounding wide marrow spaces. Proliferative changes of epiphysial cartilage, followed by ossification of the new-formed cartilage, may be seen, perhaps explaining the increase of the bone length in the so-called *type en long* of the hands.

The abnormally prominent hairiness of acromegalic patients has been mentioned. The hair is thick, the eyebrows bushy, the beard rough; the trunk, the extremities, the sometimes enlarged genitalia and the linea alba appear very hairy. In the female much hair develops on the upper lip, the chin and the cheeks, between the breasts, at the linea alba, in the groins and on the inner surfaces of the thighs and the lower parts of the legs. Almost all acromegalic patients are brunettes, while those blonds in whom acromegaly develops usually turn dark in the course of the disease.

Because in acromegaly almost all of the endocrine organs are affected, it is not strange to see signs of pluriglandular insufficiency. Besides dysfunction in the sexual sphere, disorders of sugar metabolism are common. According to Cushing and Davidoff,[12] glycosuria is found in 25 per cent and diabetes mellitus in 11 per cent of the patients, whereas Kraus quoted a frequency of 56 per cent for both. Cushing and Davidoff also reported an immediate cessation of glycosuria after removal of the eosin-

ophilic adenoma, an observation which emphasizes the effect of the eosinophilic cells on the metabolism of sugar as stressed by me more than two decades ago. (See also Yater.[14])

Occasionally myxedema may be observed, but many cases have been described in which hyperfunction of the thyroid gland has developed, characterized by increased metabolism, exophthalmos, tachycardia and tremor. The coincidence of acromegaly and goiter has been pointed out by Cushing and Davidoff in a paper illustrated with excellent pictures showing the microscopic structure of the goiter. Obesity also can be seen in some instances and may assume, though only rarely, the character of adiposogenital dystrophy. In other instances, however, after the disease has been of many years' duration the acromegalic patient passes into a cachectic stage.

The cause of acromegaly, as stressed, must be sought in hyperfunction of the eosinophilic cells, which are usually proliferated in the form of an adenoma.

A much discussed question is what part the eosinophilic adenoma, the destruction of the hypophysis and the pressure exerted on the interbrain play in the genesis of acromegaly. A case in which the eosinophilic adenoma developed within the sphenoid bone without any secondary injury to the hypophysis and the interbrain illustrates the problem at least partially. In this case, observed by Erdheim,[8] the sexual glands and their function remained normal despite the long duration of the disease, and cachexia was not present. On the other hand, acromegaly and splanchnomegaly were just as strongly developed as in other cases of acromegaly. This case permits the conclusion that the genital dystrophy and the cachexia, as well as the obesity, which are observed in some instances, are due to the destruction within the hypophysiodiencephalic system (in the hypophysis or in the interbrain or in both). They apparently are not sequels of the eosinophilic hyperpituitarism, which is responsible only for excess growth due to overproduction of growth hormone. As to the obesity, however, it must be remembered that it is rare in acromegalic patients despite the injury to the hypophysiodiencephalic system, which if caused by processes other than eosinophilic adenoma would lead to adiposogenital dystrophy. Kraus[15] tried to explain this fact by the supposition that the specific action of the eosinophilic adenoma on the pancreatic islets (antagonistic to them) lowers the tolerance for sugar, thus promoting glycosuria and diabetes mellitus but preventing obesity. This hypothesis was later supported by Houssay's experiments, which demonstrated the antagonistic relationship between the anterior lobe of the hypophysis and the islets of Langerhans.

The hypertrichosis so frequently seen in acromegalic patients has a debatable origin. Probably hyperfunction of both the hypophysis and the adrenal cortex is responsible for the abnormal hairiness. The cachexia in advanced acromegaly apparently is due to the destruction of normal hypophysial tissue by the eosinophilic tumor.

In some instances the picture of acromegaly does not appear fully developed, the enlargement being limited to certain organs. The changes in the hypophysis in the incomplete or fugitive condition (Cushing[16]) have been described differently. Some of the findings reported include mixed cell adenoma, fetal cell adenoma and enlargement of the hypophysis with increase of eosinophilic cells. Kraus is inclined to refer the acromegaloid aspect of some pregnant women to the action of the pregnancy cells, which he regards as functionally close to the eosinophilic cells. While the latter serve growth in postnatal life, the pregnancy cells seem to be destined to support the growth of the fetus.

There is a syndrome described by Brugsch,[17] called acromicria or osteogenital dystrophy, which seems to be the opposite of acromegaly. It is characterized by small stature and small fingers and toes, with the cortex of the phalanges thinned, the spongy bone rarefied and the bone poor in calcium. The skull and the mandibles may be similarly involved. Falling hair, headache, cessation of menses and tenderness of fingers and toes with acrocyanosis complete the clinical picture. Polyuria and obesity may complicate the disease. Because of lack of autopsy observations, the genesis of this disease has not been determined, but insufficiency of the hypophysis has been held responsible for it (Brugsch[17]; Parhon, Ballif and La Vrénenco[18]).

HYPOPHYSIAL GIGANTISM

Hypophysial gigantism is characterized not only by a body height greatly exceeding the average height of the respective race and by a remarkable increase in the volume of the viscera

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16. Cushing, H.: The Pituitary Body and Its Disorders, Philadelphia, J. B. Lippincott Company, 1912.

17. Brugsch, T.: Med. Klin. **23**:81, 1927.

18. Parhon, C. I.; Ballif, L., and LaVrénenco, N.: Rev. franc. d'endocrinol. **7**:307, 1929.

but also by its acromegalic aspect. This usually facilitates the differentiation between hypophysial and eunuchoid gigantism, though there are cases in which both acromegalic and eunuchoid symptoms are combined. Some of the patients are primarily eunuchoid, with acromegalic symptoms acquired at an older age. Symptoms disclosing the eunuchoid origin of the gigantism are the infantile habitus, the increase of the length of the lower over the upper segment of the body, the hypoplasia of the sexual organs, especially the gonads, the hypotrichosis, the lack of beard and the patent epiphysial lines. Contrarily, eunuchoid symptoms may appear in primarily acromegalic giants in the further course of that disease.

The close functional relationship between the hypophysis and the gonads makes it plausible that both are usually involved in either type of gigantism, the acromegalic or the eunuchoid. With overlapping symptoms, pertinent to each type, the differentiation of the two forms of gigantism and the classification of cases often may become difficult or even impossible. The relations between hypophysial gigantism and acromegaly are close, particularly inasmuch as acromegalic persons often are tall (about 20 per cent of them exceed a body length of 177 cm.), so that the border between hypophysial gigantism and acromegaly may not always be sharp.

According to Brissaud and Meige,[19] hypophysial gigantism may be nothing else than acromegaly arising before the closure of the epiphysial lines; in other words, the pathologic growth begins in the period of physiologic growing and extends into the period in which the physiologic growth normally has finished. This conception certainly is correct in all cases in which an eosinophilic adenoma developed prior to the age at which normally the body growth has ceased.

Among the cases of hypophysial gigantism noted in the literature there are not many in which the hypophysis was examined carefully. In older publications mention is made of various types of tumor of the hypophysis, such as adenoma, carcinoma, sarcoma and adenosarcoma. Furthermore, hypertrophy, sclerosis, cystic degeneration, destruction by hemorrhage and other conditions have been described as causes of gigantism.[20] In many of the cases the lesion of the hypophysis evidently was misinterpreted. From cases in which the hypophysis was carefully examined, it is known that the morphologic substratum of hypophysial gigantism is hyperplasia or adenoma of the eosinophilic cells of the hypophysis. One must regard as a reliably examined case of hypophysial gigantism that of Jedlicka,[21] which concerned a boy 14 years of age and 171 cm. tall with obesity, hypergenitalism and hypertrichosis. The autopsy revealed glioma of the cerebellum and enlargement of the pineal gland and of the hypophysis. The hypophysis showed fibroma in the pars intermedia and preponderance of the eosinophilic cells in the anterior lobe, which was twice as large as normal. The testicles contained large numbers of spermatozoa and an increase in interstitial cells, while the adrenal glands were hypoplastic.

Remarkable also is the case of Buday and Jancsó:[20e] The patient at the age of 20 years measured 163 cm. and at the age of 35 years 200 cm. At 20 years his epiphysial lines were not yet closed. He had been impotent since his third decade; the testicles were atrophic. Oddly, the authors call the tumor in the hypophysis adenosarcoma, a mistake frequently found in the old literature, in which adenoma of the hypophysis often has been interpreted as perivascular sarcoma or adenosarcoma.

In a case of Huchard and Launois,[20d] the hypophysis was found sclerotic, but the sella was enlarged, and this observation allows the conclusion that the sclerosis evidently developed within an adenoma, which in all probability was eosinophilic in nature. The abnormal growth started at the age of 12 years, and at 18 years the patient was 197 cm. tall. Gigantism and acromegaly were here combined. In another case of hypophysial gigantism, cited by Berblinger,[22] the sella was filled with fibrous tissue infiltrated by blood pigment and underlaid by a thin coat of hypophysial epithelium. Kraus interpreted this condition as eosinophilic adenoma destroyed by hemorrhage and later largely replaced by scar tissue; the patient was known to have suffered trauma. He died of tuberculosis of the lungs, as many of these giants do.

Though reliable pathologic-anatomic observations on the hypophyses of hypophysial giants are few, it might be correct to interpret this endocrine disturbance as eosinophilic hyperpituitarism which develops either primarily in youth in a heretofore normal person or secondarily, superimposed on eunuchoidism. The

19. Brissaud, E., and Meige, H.: J. de méd et chir. prat., Jan. 25, 1895.

20. (*a*) Hutchinson, W.: Am. J. M. Sc. **110**:190, 1895; (*b*) New York M. J. **72**:89 and 133, 1900. (*c*) Launois, P. E., and Roy, P.: Etudes biologique sur les géants, Paris, Masson & Cie, 1904. (*d*) Huchard, H., and Launois, P. E.: Bull. et mém. Soc. méd. d. hôp. de Paris **20**:1444, 1903. (*e*) Buday, K., and Jancsó, N.: Deutsches Arch. f. klin. Med. **60**:385, 1898.

21. Jedlicka, V.: Lek. Sborn. **25**:24, 1924.

22. Berblinger, W.: Med. Klin. **15**:1029, 1919.

underlying pathologic process in the hypophysis is hyperplasia or adenoma of the eosinophilic cells.

Falta [23] interpreted somewhat differently the genesis of the gigantism, which is so often combined with eunuchoidism, diabetes mellitus and great weakness following abnormal strength of muscle. He considered that there is an abnormal anlage of the entire endocrine system, distinguished by increase of endocrine activity in the first stage of the disease and by premature exhaustion and emaciation in the further course. In a number of cases cachexia virtually characterizes the last phase of the disease.

II

The conditions of hypophysial dwarfism, Fröhlich's syndrome and hypophysial cachexia result from total or partial deficiency of the adenohypophysis, though the important role of the interbrain in the genesis of Fröhlich's syndrome should not be underestimated.

HYPOPHYSIAL DWARFISM (NANOSOMIA PITUITARIA)

The hypophysial dwarf is a person who was born normal in size and stopped growing in childhood. The body size and proportions correspond to those of a child; the epiphysial lines stay open until an adult age; the external genitalia are markedly underdeveloped; the gonads appear hypoplastic and atrophic; beard and body hair are lacking; the skin is dry, flabby and often wrinkled, thus indicating the older age. The intelligence usually is fairly normal, though sometimes slightly below average. The blood pressure and the basal metabolism tend to be below normal.

The hypophysis in nanosomia pituitaria is usually destroyed or severely damaged by an intrasellar or an extrasellar craniopharyngioma and frequently shows marked retrogressive lesions indicating long duration of the new growth. Extensive calcification has often been observed. Hypophysial dwarfism as the result of teratoma of the hypophysis (described in the older literature several times) should be considered with caution, since old craniopharyngioma altered by retrogressive changes has often been mistaken for teratoma.

Occasionally the destruction of the hypophysis is due to a lesion other than craniopharyngioma, such as fibrosis of the anterior lobe (probably secondary to ischemic necrosis), atrophy of the anterior lobe, colloid-cystic degeneration of the anterior lobe, congenital syphilis, chronic hydrocephalus or malformation of the hypophysis.

In a report of Katzenstein [24] concerning a female dwarf 50 years old and 131 cm. tall. the sella turcica as well as the hypophysis are described as small, with the hypophysial capsule thickened, the anterior lobe fibrotic and only remnants of glandular tissue present. The lesion of the hypophysis in the presence of several stigmas of congenital hyphilis was interpreted by the author as being syphilitic.

Fink [25] described dwarfism in a 21 year old woman with chronic hydrocephalus, marked protrusion of the floor of the third ventricle, extreme atrophy of the tuber cinereum, distention of the infundibulum, optic atrophy and pressure on the hypophysis, the anterior lobe of which showed the chromophobe cells in the majority, while the posterior lobe was not altered except by edema. Another case of dwarfism due to hydrocephalus was described by Schultz.[26]

Two cases of dwarfism caused by malformation of the hypophysis have been described by Priesel [27] and Kraus,[28] respectively. In these 2 cases, however, the clinical and anatomic features differed from those of classic hypophysial dwarfism. In both cases the epiphysial lines were closed. In Priesel's case, which concerned a man 91 years old and 132 cm. tall, the dwarfism was caused by tuberal dystopy of the posterior lobe, the type which is characterized by the posterior lobe being amalgamated to the base of the interbrain while the anterior lobe is located in the sella turcica as usual. In this case the intrasellar part of the hypophysis was transformed into a thin-walled cyst extending to the sphenoid sinus. In the wall of the cyst a thin layer of the parenchyma of the anterior lobe was present. The craniopharyngeal canal was patent; the posterior lobe lay outside the sella at the cerebral base behind the optic chiasm. Kraus's case concerned a 26 year old jockey 130 cm. in height and proportional in structure. The face was that of a child, but with fine wrinkles about the mouth and the eyes. There was no hair in the axillas, and the fine hair at the mons veneris showed feminine distribution. The epiphysial lines were closed. The testicles were large and histologically normal. The

23. Falta, W.: Erkrankungen der Blutdrüsen, Berlin, Julius Springer, 1913.

24. Katzenstein, R.: Virchows Arch. f. path. Anat. **289**:222, 1933.

25. Fink, E. B.: Arch. Neurol. & Psychiat. **17**:332, 1927.

26. Schultz, A.: Virchows Arch. f. path. Anat. **248**: 180, 1924.

27. Priesel, A.: Beitr. z. path. Anat. u. z. allg. Path. **67**:220, 1920.

28. Kraus, E. J.: Virchows Arch. f. path. Anat. **286**: 656, 1932.

hypophysis, weighing 0.29 Gm. only, offered the typical picture of dystopia infundibularis of the posterior lobe. While the eosinophilic and chief cells were numerous, the basophils were decreased in number. The pars intermedia with the typical colloid cysts was absent. In both cases the clinical and anatomic picture of the dwarfism was not complete; nevertheless, on account of the severe lesion of the hypophysis as the only probable cause, these cases must be considered instances of hypophysial dwarfism. Apparently, the injury of the hypophysis was not marked enough to eliminate the entire function of the organ but was severe enough to produce at least a forme fruste of hypophysial dwarfism. It is known that by slight injury of the hypophysis growth in length is not caused to cease entirely but is only retarded; the bone nuclei appear much later than they normally should, resulting in deferred closure of the epiphysial lines. This explains why some hypophysial dwarfs exhibit ossification of their epiphysial lines, as in the 2 cases mentioned.

The changes in the testicles in typical cases of hypophysial dwarfism (which as a rule involves only males) are not quite uniform, depending on the age at which the destructive process in the hypophysis begins. The final picture is a summation of hypoplasia and atrophy. In younger patients the hypoplasia usually is more distinct; in older ones the atrophic lesions are predominant. The most characteristic feature shown by the testicles in hypophysial dwarfism is the lack of Leydig's interstitial cells, in contrast to other types of atrophy of the testicles. The lack of interstitial cells, however, is also seen in adiposogenital dystrophy and is characteristic of testicular atrophy of hypophysiodiencephalic origin (Berblinger [29]; Kraus [15]).

The dwarfism observed by Wells [30] in a 72 year old woman, 124.5 cm. tall, with cystic degeneration of the anterior lobe, does not represent the hypophysial type. The absence of the thyroid gland, with only a lingual goiter present, together with symptoms of cretinism, characterizes this patient as a hypothyreotic dwarf with secondary colloid degeneration of the anterior lobe, a condition often observed in cretins.

A case difficult to classify has been described by Kraus.[31] The patient was an idiotic midget, 121 cm. tall, with marked adiposity. In addition to various anomalies of the brain, such as hypoplasia of the right cerebral hemisphere and microgyria, there was a failure of development in the hypophysis, which weighed only 0.16 Gm.; this is exceedingly small in comparison with the normal weight of about 0.65 Gm. The posterior lobe was tiny, and the anterior lobe was characterized by a striking lack of eosinophilic cells and one microscopically small basophilic adenoma. The skeleton was proportional, and the epiphysial lines were ossified. Evidently the idiocy accompanied by various anomalies of the brain, including the small size of the neurohypophysis, was the basic condition. The marked hypoplasia of the neurohypophysis might have caused the atrophy of the anterior lobe, which in turn led to the dwarfism, particularly since the eosinophilic cells were few. Since not only separation but a defective connection of the hypophysis and the brain results in atrophy of the anterior lobe, the interpretation given seems to be justified.

In hypophysial dwarfism the thyroid gland, among other endocrine organs, shows atrophy, while the parathyroid glands usually do not appear affected. The adrenal glands are conspicuously small.

The skeleton, characterized by small size of the bones and open epiphysial lines, gives the impression of having been arrested in its infantile state. There is a marked difference, however, between the endochondral ossification zone of a child and that of a hypophysial dwarf. While in a child all processes of bone growth are in full swing, those in a hypophysial dwarf appear completely arrested, the spongy bone being separated from the cartilage by an uninterrupted plate of bone (Erdheim [32]). Because of the lack of growth hormone, the proliferation of cartilage does not receive any stimulation, and therefore the bones do not grow, and since vascular erosion and formation of bone tissue do not take place, the epiphysial lines do not disappear. Necrosis of the epiphyses has been observed repeatedly. The degree of dwarfism depends on the age when the destructive lesion of the hypophysis began and on the severity of the lesion. The farther back in youth the injury of the hypophysis goes, the more pronounced is the dwarfism. Therefore, it seems justifiable to distinguish three types of hypophysial dwarfism: (1) nanosomia congenitalis, the destruction of the hypophysis being assumed to have begun in intrauterine life; (2) nanosomia infantilis; (3) nanosomia tarda, the latter mainly observed as a part of adiposogenital dystrophy (Erdheim [32]). In the third type the tumor

29. Berblinger, W.: Beitr. z. path. Anat. u. z. allg. Path. **87**:233, 1931.

30. Wells, H. G.: Arch. Path. **20**:64, 1935.

31. Kraus, E. J.: Beitr. z. path. Anat. u. z. allg. Path. **65**:535, 1918.

32. Erdheim, J.: Beitr. z. path. Anat. u. z. allg. Path. **62**:302, 1916.

has injured not only the anterior lobe but the base of the diencephalon, and it is the lesion of the latter which chiefly causes the obesity. The fact that the adult hypophysial dwarf, as a rule, is not obese is explained by the destruction of the anterior lobe, since loss of function of the anterior lobe causes cachexia, which counteracts the development of the obesity produced by injury of the interbrain. In some instances hypophysial dwarfism may be associated with diabetes mellitus (Taylor [33]) or diabetes insipidus.

As to the genesis of nanosomia pituitaria, there is no doubt that the lack of function of the anterior lobe is responsible for the arrest in body growth as well as in sexual development. While the lack of eosinophilic cells causes the disturbance of growth, the lack of basophilic cells seems to be accountable for the genital dystrophy. Some authors have separated hypophysial infantilism from hypophysial dwarfism, though they do not deny the close pathogenetic relationship, with transition of one into the other. Hypophysial infantilism differs chiefly from the other by the fact that the retardation in growth and the arrest in development are transient. In fact, there is only a difference of grade between hypophysial infantilism and dwarfism. The involvement of the hypophysis in hypophysial infantilism is, of course, less severe than that in the classic hypophysial dwarfism. In 1 case Berblinger [34] found the hypophysis smaller than normal and poor in chromophilic cells. Unfortunately, there are not many cases of this type in which the hypophysis has been morphologically examined with the necessary care.

There seems to be a certain relation between hypophysial dwarfism and a peculiar disease described by Gilford [35] and called by him progeria. The patients are dwarfs. The skull is large and the face small; the fontanels are open; the head is bald; the eyes are large and protruding; the lashes and brows are almost absent; the nasal cartilage is prominent; the teeth are few and irregular; the lower jaw is small. The clavicles and scapulas are small; the abdomen is distended; the epiphyses are enlarged; the nails are atrophic; the skin is tense and contains brown pigment. Gilford characterized one of his patients, a boy 5 years old, as a "wizened dwarfish old man." Virtually these juvenile patients exhibit certain characteristics of an old person and others of a young person. Though several cases have been observed and reports published, descriptions of observations made at autopsies are lacking.[36]

FRÖHLICH'S SYNDROME (DYSTROPHIA ADIPOSOGENITALIS)

In 1901 Fröhlich first recognized the causal connection between lesions of the hypophysis and the syndrome which is named after him.[37] The condition is characterized mainly by obesity of the eunuchoid type, by atrophy of the gonads and genitalia, impotence, cessation of menses and sterility, loss of secondary sex characteristics and sluggishness of metabolism. Headaches, vomiting, visual disturbances, changes in temperature, pulse and blood picture, mental disturbances, somnolence and often polyuria complete the clinical picture. Beginning in youth before the closure of the epiphysial lines and sexual maturity, the condition assumes an aspect which represents a combination of obesity and dwarfism. No particular age is involved but the majority of the patients are middle aged and the syndrome is seen somewhat more frequently in women than in men.

The changes causing adiposogenital dystrophy are exceedingly variable in nature, origin, topography and extension, but common to all cases is the location within the hypophysiodiencephalic system. It might be practicable to set up four groups of cases depending on the types of lesions leading to dystrophia adiposogenitalis. The first group comprises cases with the destructive lesion limited to the interbrain; the second, cases with interruption of the continuity between the anterior lobe and the interbrain as a result of partial or total destruction of the neurohypophysis. Cases with the primary lesion limited mainly to the hypophysis form the third group, and cases with involvement of the entire hypophysiodiencephalic system constitute the fourth group.

Lesions of the interbrain underlying adiposogenital dystrophy are, among others, suprasellar craniopharyngioma, glioma, meningioma, cholesteatoma and exostoses of the dorsum sella turcicae or of the posterior clinoid processes which exert pressure on the region of the third ventricle. Tumors in the region of the third ventricle play an especially important part (Fulton and

33. Taylor, N. M.: Endocrinology **22**:115, 1938.

34. Berblinger, W., in Hirsch, M.: Handbuch der inneren Sekretion, Leipzig, C. Kabitzsch, 1932, vol. 1, p. 1023.

35. Gilford, H.: Practitioner **73**:188, 1904.

36. Variot and Pironneau: Bull. Soc. de pédiat. de Paris **12**:431, 1910. Rand, C. W.: Boston M. & S. J. **171**:107, 1914. Curtin, V. T., and Kotzen, H. F.: Am. J. Dis. Child. **38**:993, 1929. Gilford.[35]

37. Fröhlich, A.: Wien. klin. Rundschau **15**:883, 1901.

Bailey [38]). Furthermore, chronic tuberculosis, syphilitic meningoencephalitis, epidemic encephalitis and chronic hydrocephalus are accountable for the disease.

The chronic hydrocephalus causes extension of the floor of the third ventricle, which when the condition is severe bulges out from the base of the brain like a thin-walled cyst. Depletion of ganglion cells in the nucleus paraventricularis and accumulation of lipoid in the cells of the nucleus basalis and the nucleus tuberis have been observed. In cases of severe and long-lasting hydrocephalus the hypophysis shows depletion of the chromophilic cells in the anterior lobe and finally atrophy of the whole organ. In cases of adiposogenital dystrophy secondary to epidemic encephalitis, an injury of the vegetative centers in the floor of the third ventricle has been held responsible for the development of the disease. A rare cause of adiposogenital dystrophy is a chronic, sclerosing type of encephalitis limited to the infundibulum, the tuber cinereum and the corpora mammillaria, apparently developing after trauma of the skull, as described by Kraus.[39]

Adiposogenital dystrophy caused by separation of the anterior lobe from the interbrain after destruction of the neurohypophysis or parts of it is rare. Tuberculosis of the infundibulum (van Valkenburg [40]), extreme hypoplasia of the posterior lobe (Kraus [15]), gliosis of the posterior lobe, cysts with a large accumulation of colloid in the pars intermedia (separating both lobes), compression by a fibroma of the dura mater (Askanazy [41]) and other pathologic conditions have been found as underlying lesions. In rare instances trauma is responsible for the disruption of the hypophysiodiencephalic system. For instance, an injury by a shot was followed by replacement of the posterior lobe and hypophysial stalk with scar tissue (Maranon and Pintos [42]). A fracture of the skull base and necrosis of the stalk secondary to a fall resulted not only in adiposogenital dystrophy but in diabetes mellitus (Verron [43]).

More frequent is adiposogenital dystrophy with the destruction involving the hypophysis itself. Chromophobic (sometimes psammous) and basophilic adenoma, rarely eosinophilic adenoma, intrasellar and exceptionally intrasphenoidal craniopharyngioma, angioma, angiocavernoma and glioma of the posterior lobe are the tumors found in cases of this type. In addition tuberculosis, syphilis (interstitial hypophysitis, especially in cases of congenital syphilis, or more often gumma), colloid cysts in the pars intermedia, hypoplasia of the hypophysis, traumatic injuries with hemorrhage, old cystic hematoma apparently traumatic in nature, compression of the hypophysis by sphenoidal empyema, chronic abscess involving the entire hypophysis, fibrosis of the anterior lobe and other conditions are listed as present in cases of this type of adiposogenital dystrophy. In a Russian Jew, whose weight was 450 pounds (204 Kg.) and who suffered from somnolence resembling narcolepsy, polyuria, hypertension and genital atrophy and had increased tolerance for sugar, de Santo [44] found a large cyst of the posterior lobe filled with debris.

In the majority of cases of adiposogenital dystrophy the destruction involves the hypophysis as well as variable parts of the interbrain. The destructive lesions include tumors of the hypophysis, such as simple and cancerous adenoma and craniopharyngioma, specific granuloma, such as that of tuberculosis and gumma, tumors of the brain, meningioma and cholesteatoma of the cranial base or third ventricle with devastating compression of the hypophysis.

Aside from the causal process within the hypophysiodiencephalic system, secondary changes involving the brain, the cranial nerves and the skull may be seen in patients with adiposogenital dystrophy. It is known that tumors of the hypophysis, intrasellar as well as extrasellar, cause signs of increased intracranial pressure relatively late. When a considerable increase of the pressure within the brain has been present, the brain shows such abnormalities as swelling, flattening of the convolutions, narrowing of the sulci and cerebral hernias. These changes are much more severe if the adiposogenital dystrophy is caused by tumor of the interbrain (for instance, glioma) than if it is due to tumor of the hypophysis itself.

The pressure exerted by the intrasellar or the extrasellar tumor of the hypophysis against the optic chiasm leads to displacement, flattening and distention of the chiasm, followed by atrophy of the nerve fibers, which causes visual disturbances and even total amaurosis. Injury to the oculomotor and trochlear nerves is responsible for the paralysis of ocular muscles. The changes of the sella turcica are variable, according to the

38. Fulton, J. F., and Bailey, P.: J. Nerv. & Ment. Dis. **69**:1, 1929.

39. Kraus, E. J.: Med. Klin. **22**:485, 1926.

40. Van Valkenburg: Nederl. tijdschr. v. geneesk. **64**:997, 1920.

41. Askanazy, M., cited by Kraus.[15]

42. Maranon and Pintos: Nouv. iconog. de la Salpêtrière **28**:185, 1916.

43. Verron, O.: Centralbl. f. allg. Path. u. path. Anat. **31**:521, 1921.

44. De Santo, D. A.: Arch. Path. **16**:760, 1933.

site, the size and the duration of the destructive process. Slight enlargement of the hypophysis widens the sella without considerable involvement of the sellar entrance. A larger intrasellar tumor widens and deepens the sella remarkably; the osseous floor of the sella becomes destroyed; the dorsum sellae becomes atrophic, bowed backward and partially destroyed; the tuberculum sellae may disappear entirely. With a suprasellar tumor, usually the sella is not deepened so much, but the entrance to the sella is greatly widened. The floor of the sella may be destroyed; the dorsum and the posterior clinoid processes are atrophic; the tuberculum sellae vanishes (Berblinger [34]).

Shelden [45] instructively described the changes due to pressure by a tumor on the confines of the sella turcica as well as on the visual pathways. The character of the visual defect depends much on the topography of the optic chiasm and on the direction of growth of the tumor. Shelden reproduces pictures which he credits to de Schweinitz,[46] showing the anatomic variation in the site of the optic chiasm. The chiasm may be located far forward or far backward or in intermediate positions relative to the sella. The farther back it is located, the longer must be the optic nerves. These different topographic conditions explain the different visual defects. (For details see the original paper.)

The changes of the base of the skull imprint certain characteristics on the roentgenogram. According to Raab,[47] with an intrasellar tumor the dorsum sellae is bowed backward in addition to showing marked erosion, while the limbus forms a sharp edge. The suprasellar tumor, however, is distinguished by dilatation of the sellar entrance and erosion of the tuberculum sellae as well as of the dorsum sellae from above. (See also Kornblum and Osmond.[48]) Henderson [49] subjected 367 pertinent cases to an analysis of disorders and made the interesting observation that sexual dysfunction develops only after the sella turcica has become considerably expanded.

The obesity in adiposogenital dystrophy is characterized mainly by the distribution of the fat tissue with predilection particularly for the neck, the chin, the shoulders, the breasts, the lower part of the abdomen and the hips. This distribution of the fat masses which do not infiltrate the musculature is, of course, more striking in males than in females. It does not disappear even when the patient, for any reason, becomes emaciated.

Aside from occasional osteoporosis the bones do not show changes except in those cases in which the disturbance has started before the closure of the epiphysial lines. In such cases various degrees of dwarfism result. According to Berblinger [29] and Kraus,[28] in these cases the anterior lobe, which furnished the growth hormone, need not be destroyed, for the separation of the hypophysis from the interbrain is sufficient to cause retardation or even complete stoppage of growth.

Typical is the loss of pubic as well as axillary hair, and in males the loss of beard on lips and cheeks.

The testicles show hypoplasia in those cases in which the adiposogenital dystrophy started in youth, with the germinal tissue underdeveloped and the interstitial tissue increased. In cases in which the adiposogenital dystrophy began after sexual puberty had been reached, the testicles undergo atrophy. There is no spermatogenesis, and the atrophy of the germinal tissue is accompanied by thickening and hyalinization of the membrana propria of the seminiferous canaliculi; Leydig's interstitial cells are sparse or entirely lacking, as is typical of cases with hypophysiodiencephalic lesions. In females the ovarian follicles disappear or the ovaries show cystic degeneration with cessation of menses and resulting sterility. Simultaneously with hypoplasia or atrophy of the gonads, other parts of the sexual apparatus, such as the uterus, the fallopian tubes, the epididymis, the prostate and the seminal vesicles, exhibit changes either of underdevelopment or of atrophy, depending on the age at which the disease began. According to Henderson,[49] the injury which the space-occupying lesion inflicts on the basophilic cells, which allegedly are the producers of the gonadotropic hormone, is responsible for the loss of sexual function.

Not all features in the picture of adiposogenital dystrophy are equally developed. Adipositas, genital dystrophy, impotence and hypotrichosis may vary considerably as to their degree, and one or more of the characteristic symptoms, particularly the genital dystrophy or the hypotrichosis, may be absent in some cases. If obesity rules the clinical picture, with the other symptoms less definite, frequently the term "cerebral obesity" is used. The following cases may illustrate the conditions:

Kraus [50] examined the body of a 40 year old man with marked obesity, loss of libido sexualis and impotence. A huge mixed cell adenoma chiefly made up

45. Shelden, C. H.: M. Clin. North America **24**:981, 1940.

46. de Schweinitz, G. E., cited by Shelden.[45]

47. Raab, W., cited by Berblinger.[34]

48. Kornblum, K., and Osmond, L. H.: Ann. Surg. **101**:201, 1935.

49. Henderson, W. R.: Endocrinology **15**:111, 1931.

50. Kraus, E. J.: Med. Klin. **20**:1290 and 1328, 1924.

of chromophobes had destroyed the interbrain and separated the hypophysis from it. The hypophysis was found compressed and diminished in size at the bottom of the dilated sella, but all three cell types of the anterior lobe were fairly well preserved. The testicles together weighed 40 Gm. and showed abundant spermatogenesis, with many spermatozoa crowded in the seminal vesicles. Despite normal testicles, the man was impotent for one year. There was no loss of hair; the beard, as well as the pubic and the axillary hair, were normal.

In another case a man 32 years old gained 25 pounds (11 Kg.) in one year because of a cholesteatoma of the third ventricle, which led to marked hydrocephalus. The anterior lobe of the hypophysis was hyperplastic, owing evidently to the long-lasting intracranial pressure. The testes were normal in size and showed spermatogenesis preserved; nevertheless, libido sexualis was lessened.

Very instructive is a case observed by Kraus, concerning a man 36 years of age. Chronic sclerosing encephalitis limited to the floor of the third ventricle caused severe obesity of the eunuchoid type; the hypophysis was diminished in size (0.37 Gm.), but all three cell types were represented, the chromophilic cells being somewhat smaller than normal. The beard was a little scanty around the angles of the mouth and on the chin; the testicles were normal in size, and the spermatogenesis was unchanged, but Leydig's interstitial cells were conspicuously rare. In the period prior to death the patient was entirely impotent.

Cases like these, by no means rare, illustrate the importance of the vegetative centers of the interbrain in connection with the genesis of the obesity, since in all these cases the injury to these centers of the interbrain was much more severe than the lesion of the hypophysis. But it would be wrong to believe that the obesity is a specific diencephalic symptom. Though in many cases marked obesity can be referred to the destruction of the interbrain proper, there are many other cases of adiposity with the hypophysis primarily and chiefly involved. Since in some of these cases the destructive process is strictly limited to the hypophysis without injury to the interbrain, a hypophysial origin of the obesity also must be admitted. Changes limited to the hypophysis itself without anatomic involvement of the interbrain include necrosis of the anterior lobe, sclerotic atrophy of the anterior lobe, cyst in the pars intermedia (separating the two lobes) and a small neoplasm of the hypophysis without protrusion into the cranial cavity. In conformity with this assertion is a statement of Cushing [51] that the obesity is more developed in patients in whom a tumor—for instance, craniopharyngioma—has injured the cerebral centers than in those with a strictly intrasellar lesion, but that also in the latter appreciable adiposity may be present. He also found adiposity in patients with chromophobe adenoma, in whom there was no possibility of a tuberal injury. A review of the clinical histories of these patients revealed that in about 60 per cent of the females a sudden increase of the weight had occurred long before the evidence of chiasmal pressure. Looking on the hypophysis and the interbrain as a functional unit, one should not be amazed to see the same symptom, though different in severity, caused by destruction of the interbrain as well as by destruction of the hypophysis.

In some cases of adiposogenital dystrophy, obesity is lacking, and the picture is ruled chiefly by the genital dystrophy. For instance, Kraus [50] examined the body of a man 44 years of age with the floor of the third ventricle entirely destroyed by a suprasellar craniopharyngioma. There was no obesity, but the testicles were extremely fibrotic, without any interstitial cells, as is typical of cases with a hypophysiodiencephalic lesion. The hypophysis was diminished in size and flat and showed decrease in number and size of the chromophilic cells.

Kraus [50] tried to explain the lack of obesity in some of these cases by the atrophy and degeneration of Langerhans' islands, which might have caused a decrease in the carbohydrate tolerance, thus counteracting the development of obesity. Indeed, hypopituitarism may be associated with diabetes mellitus as shown by John [52] and others. The decrease in sugar tolerance seems to be the reason why, as a rule, obesity is lacking in acromegaly despite destruction of the hypophysis and often of the interbrain. Virtually about 56 per cent of all acromegalic patients have diabetes mellitus or glycosuria, due to the eosinophilic adenoma, which causes an injury of Langerhans' islets with a breakdown of their function. In a case described by Kraus and Reisinger,[53] the hypophysis was greatly damaged because of being compressed by a chromophobe adenoma. The pancreas showed lipomatosis and atrophy, with the islands decreased in number, partially degenerated and atrophic. Despite the marked injury of the hypophysis, no obesity had developed.

In other cases the lack of function of the anterior lobe, known as the cause of hypophysial cachexia, counteracts the development of obesity or causes the obesity to recede or disappear entirely, and after many years' duration of the adiposogenital dystrophy even cachexia may develop. Two cases of adiposogenital dystrophy passing into cachexia have been examined by me.

1. A man 42 years of age had obesity, genital dystrophy and hypotrichosis due to a huge psammous

51. Cushing, H.: Lancet **2**:119 and 175, 1930.

52. John, H. J.: Endocrinology **9**:397, 1925.

53. Kraus, E. J., and Reisinger, A.: Frankfurt. Ztschr. f. Path. **30**:68, 1924.

adenoma of the hypophysis. Finally, after he had been ill for several years, hypophysial cachexia developed. The hypophysis had been entirely destroyed and the interbrain crowded out and greatly damaged by the tumor.

2. A man 36 years of age had obesity, genital dystrophy and hypotrichosis warranting the diagnosis of adiposogenital dystrophy. After seven years he died of cachexia. The floor of the third ventricle had been destroyed by a huge glioma; the hypophysis was markedly compressed but histologically was not too severely damaged.

It is hard to say whether the hypophysial disorder or the long-lasting intracranial pressure in these and similar cases is accountable for the cachexia. It is known that tumors of the brain frequently cause cachexia after years of duration without any evidence of hypophysial origin.

As said, more than one typical symptom of the adiposogenital dystrophy may be absent in certain cases. Such a case has been reported by Kraus:[28] A man 21 years of age died of a huge suprasellar craniopharyngioma. No obesity was present, and the spermatogenesis was partially preserved. The hypophysis was atrophic, weighing only 0.47 Gm., and showed necrotic areas and some fibrosis in the anterior lobe, but the cells, particularly the chromophilic cells, though fewer, were not essentially altered. In another case[50] hypotrichosis in a man 36 years of age was the only noticeable endocrine symptom. There was no obesity and no impotence, the testicles showing spermatozoa, though these were decreased in number. Nevertheless, the lesion was of the type which usually causes the complete picture of adiposogenital dystrophy. The floor of the third ventricle was destroyed by a glioma; the hypophysis was compressed and flattened by the tumor, but the cells of the anterior lobe were relatively fairly well preserved.

As the term "adiposogenital dystrophy" seems to be unsuitable for cases of obesity without genital dystrophy, just as it is for cases with genital dystrophy without obesity, it might be advisable to use in these incomplete cases the names "hypophysiodiencephalic adiposity" and "hypophysiodiencephalic genital dystrophy." The great variety in the clinical picture of adiposogenital dystrophy becomes better understandable when the hypophysis and the interbrain are regarded as a functional unit with close dependence of one on the other. This close relationship is proved not only clinically and experimentally but also morphologically, since changes in one part inevitably are followed by secondary changes in the other part. Any severe destruction within the hypophysiodiencephalic system can cause the disease, the clinical picture of which may vary as to symptoms and their intensity, which depend on the site of the destructive process, its nature, extension and rapidity of development, its duration and, last, on the age and the sex of the patient. But even extensive injury to the hypophysis and the interbrain need not be followed by particular endocrine disturbances, as shown by Long and others.

SIMMONDS' DISEASE (HYPOPHYSIAL CACHEXIA)

Simmonds[54] is credited with the discovery that lack of function of the anterior lobe of the hypophysis is followed by cachexia and finally death in coma. The case on which he based his opinion concerned a 46 year old woman in whom, after her fifth delivery, puerperal sepsis developed, from which she never recovered. Autopsy revealed absence of the posterior lobe. The pars intermedia was represented by a few small colloid-filled cysts, while the anterior lobe was converted into scar tissue containing a few strands of atrophic epithelium. Taking the patient's history into consideration, Simmonds interpreted the condition of the hypophysis as the result of necrosis due to bacterial embolism.

The clinical picture of the disease today generally called Simmonds' disesae is characterized by chronic cachexia, a look of old age, a wrinkled face, dry skin, falling hair and teeth, cessation of menses and loss of hair in the axillary and pubic regions, as well as diminution in size of inner organs (splanchnomicria). In addition there are mental changes, such as apathy, loss of spiritual activity, drowsiness, confusion, hallucination, delirium, dizziness, spells of fainting and convulsions. The patients, the majority of whom are women, succumb to cachexia or intercurrent disease. Although in some cases progressive cachexia develops in an early stage, causing death in a short time, in the majority of cases it sets in relatively late, often killing the patient several years after the destruction of the hypophysis has occurred.

Underlying the disease is a destruction of the hypophysis or of its anterior lobe, which may be caused by different processes. In 4 of Simmonds' own cases, fibrous atrophy of the anterior lobe was found, whereas the posterior lobe was normal or slightly diminished in size. Only in 1 case (his first) was practically the entire organ destroyed. Usually the size of the organ is markedly decreased, and weights of 0.3 down to 0.15 Gm. have been recorded. In the majority of cases the disease starts secondary to childbirth, often that complicated by septic infection. Thi

54. Simmonds, M.: Deutsche med. Wchnschr. **40**: 322, 1914.

led not only Simmonds but many others to the belief that the necrosis preceding the fibrosis of the organ is embolic in nature. Today the consensus is that thrombosis is the more frequent cause of the hypophysial necrosis, based on the fact that in a great many cases no cause of embolism could be discovered. Many of the patients had severe hemorrhages during or after delivery. Therefore, severe anemia with vascular collapse is held responsible for the thrombosis in the hypophysis which, in turn, causes the necrosis. The thrombosis might arise on the first day of delivery.[55]

Tumors of the hypophysis also are important in the etiology of hypophysial cachexia. Simmonds himself described 2 cases of basophilic adenoma with extensive destruction of the hypophysis. More often chromophobe adenoma and craniopharyngioma have been observed.[56] Particularly in cases of chromophobe adenoma, symptoms may be found identical with those in Simmonds' disease, such as cachexia, low metabolism, hypotony, subnormal temperature, atrophy of skin and hair, sexual insufficiency, increase in carbohydrate tolerance and somnolence.[57]

A rare cause of the disease is a cyst in the posterior lobe [57c] or a colloid cyst in the pars intermedia separating the two lobes, with secondary depletion of the chromophilic cells, especially the eosinophils. Complete lack of eosinophilic cells together with mild interstitial fibrosis of the hypophysis was observed in a case of Simmonds' disease by Doane, Blumberg and Teplick.[58] Specific granuloma, such as gumma or that of actinomycosis, may produce hypophysial cachexia. Gumma has been described several times as the cause of the disease, particularly in regard to women. A patient with a gumma in the hypophysial stalk causing cachexia was observed by Jaffé.[59] In another patient, a 55 year old syphilitic man, this author found a hypophysis that weighed not more than 0.035 Gm. and showed marked atrophy of the anterior lobe and focal proliferation of scar tissue. Fibrosis and atrophy of the anterior lobe in syphilitic persons with cachexia have been repeatedly interpreted as the result of a specific lesion. Clinically, the favorable effect of antisyphilitic treatment suggests the syphilitic nature of the hypophysial cachexia (Riecker and Curtis [57c] and others). Compared with syphilis, tuberculosis represents a rarer cause of hypophysial cachexia. A case in which the anterior lobe was entirely destroyed by tuberculous granulation tissue was described by Kraus.[60] In some instances in which the anterior lobe is converted into a fibrous mass with calcium deposits it might be difficult, if not impossible, to differentiate between healed tuberculosis and healed gumma.

Nonspecific inflammatory lesions cannot be regarded as demonstrated causes of Simmonds' disease, though they cannot be ruled out. This is true of diffuse inflammations as well as abscesses. Other rare lesions responsible for hypophysial cachexia are an old encapsulated hematoma and traumatic injuries involving the hypophysis, such as a fracture of the cranial base causing hemorrhage in the hypophysis.[61] As a rare event, Werner, Blakemore and King [62] recently described Simmonds' disease due to an aneurysm of the internal carotid artery which was progressive despite ligation of both common carotid arteries and which destroyed the anterior lobe.

Not only the destruction of the hypophysis but apparently also the destruction of the interbrain by a glioma or a craniopharyngioma may be followed by Simmonds' cachexia. Here, of course, it is not quite obvious whether the loss of the vegetative centers of the interbrain or the separation of the hypophysis from the brain, leading to atrophy of the anterior lobe, is responsible for the cachexia. There are cases known, 1 of them described by Berblinger,[56c] in which a craniopharyngioma was located in the infundibular region, crowding out the floor of the third ventricle and rendering the hypophysis flat without destroying its structure.

In some cases of hypophysial destruction, the complete picture of Simmonds' disease does not develop; certain symptoms, such as amenorrhea or falling hair, are not present or the emaciation does not reach the excessive degree found in classic cases. In other cases, however, cessation of the menses and striking falling of the hair are the only changes that call the attention of the physician to the hypophysial involvement. These

55. Sheehan, H. L.: J. Path. & Bact. **45**:189, 1937; Lancet **2**:321, 1940.

56. (*a*) Sainton, P., and Rathery, F.: Bull. et mém. Soc. méd. d. hôp. de Paris **25**:647, 1908. (*b*) Farber, J. E.: Ann. Int. Med. **13**:217, 1940. (*c*) Foley, M. P., and Snell, A. M.: Am. J. M. Sc. **198**:1, 1939. (*d*) Jedlicka.[21] (*e*) Berblinger,[34] p. 910.

57. (*a*) Dott, N. M., and Bailey, P.: Brit. J. Surg. **13**:314, 1925. (*b*) Davidoff, L. M.: Endocrinology **10**: 461, 1926. (*c*) Riecker, H. H., and Curtis, A. C.: J. A. M. A. **99**:110, 1932. (*d*) Cushing.[16]

58. Doane, T. C.; Blumberg, N., and Teplick, G.: Endocrinology **27**:766, 1940.

59. Jaffé, R.: Frankfurt. Ztschr. f. Path. **27**:324, 1922.

60. Kraus, E. J.: Klin. Wchnschr. **16**:1528, 1937.

61. Reverchon, L.; Worms, G., and Rouquier: Presse méd. **29**:741, 1921. Munslow, R. A.; Haymond, J. L., and Crawford, A. S.: Arch. Path. **34**:431, 1942.

62. Werner, S. C.; Blakemore, A. H., and King, B. G.: J. A. M. A. **116**:578, 1941.

incomplete or fugitive forms are sometimes hard to explain, particularly in view of the extensive destruction of the anterior lobe found in some of them. In a few cases of Simmonds' disease due to destruction of the hypophysis, the interbrain was examined microscopically, and glia proliferation and degenerative changes of the ganglion cells were noted. Pathologic observations in 41 cases of Simmonds' disease were compiled by Silver.[63]

Aside from the hypophysis, many other organs are involved by atrophy in Simmonds' disease, as the liver, the spleen, the pancreas, the thyroid gland, the adrenal glands and the gonads. The histologic picture shows atrophy with or without thickening of the interstitial connective tissue. The thyroid gland particularly exhibits fibrous atrophy, round cell infiltration and chronic interstitial thyroiditis. (See also Rose and Weinstein.[64]) In the ovaries, the primordial follicles are lacking and there is no ripening of follicles. The uterus and the breasts are atrophic. The testicles do not show spermatogenesis, the walls of the canaliculi are hyalinized, and even complete fibrous hyaline destruction occurs. As disclosed by the literature, not only adults are involved by Simmonds' disease but also, though only in rare cases, children.

Gilford,[65] Hutchinson [66] and Variot and Pironneau [36] described patients characterized by dwarfism, extreme emaciation, defective hair on head and body, dryness of skin and eagle nose (due to insufficient development of the jaws), as well as hypoplasia of the genitals. (See the section entitled "Hypophysial Dwarfism.") Unfortunately, these patients were not examined thoroughly enough to clarify the genesis of this rare syndrome, which might be related to hypophysial cachexia and hypophysial dwarfism as well.

Though diabetes insipidus does not belong to the picture of Simmonds' disease, in rare instances it is encountered, as in a case reported by Kraus.[31] The patient, a man 21 years of age, had a suprasellar craniopharyngioma causing pressure atrophy of the hypophysis, resulting in extreme cachexia. It is not necessary to mention that cachexia has developed in many cases of advanced adiposogenital dystrophy, acromegaly, gigantism and hypophysial dwarfism as the result of long-lasting lack of hypophysial function.

Hypophysial cachexia constitutes an important component of a syndrome first described by Claude and Gougerot [67] and called pluriglandular endocrine insufficiency or multiple sclerosis of endocrine glands (Falta [23]). This syndrome is caused by fibrous atrophy of several endocrine glands, simultaneously involved. As a rule, the hypophysis, the thyroid gland, the adrenal glands and the gonads are involved. Accordingly, the patients offer a great variety of endocrine symptoms, including signs of hypophysial insufficiency, such as cachexia, hypothyroidism, evidence of Addison's disease and late eunuchoidism.

In the literature the disease has been frequently confused with the cachexia of Simmonds' disease, and there is some resemblance between the two diseases; however, there is one fundamental difference. In Simmonds' disease, the primary lesion concerns only the hypophysis, and the changes in the other endocrine organs with their respective symptoms are secondary in nature, dependent on the hypophysial lesion, whereas in Falta's multiple endocrine sclerosis the pathologic process involves several glands of the endocrine system primarily and simultaneously. The process is characterized by atrophy of the involved glands with subsequent fibrosis.

It must be admitted that clinically the differentiation between multiple endocrine sclerosis and Simmonds' disease may be difficult; a favorable effect of hypophysial medication supposedly speaks in favor of Simmonds' disease. Certainly, all cases in which lesions such as tumor, granuloma, ischemic necrosis and hematoma primarily involve the hypophysis while the other organs merely show secondary atrophy represent Simmonds' disease. In a case of multiple endocrine sclerosis cited by Berblinger,[56e] fibrosis and a striking scarcity of chromophilic cells were observed in the anterior lobe, while the pars intermedia, the posterior lobe and the hypophysial stalk were replaced by hyalinized fibrous tissue. The adrenal glands, the thyroid gland and the testes likewise offered the picture of fibrous atrophy.

In some cases of multple endocrine sclerosis cachexia cannot be observed. Castleman and Hertz [68] reported a case complicated by myxedema. The anatomic changes consisted of fibrosis of the anterior lobe of the hypophysis, extreme atrophy and fibrosis of the thyroid gland and atrophy and lipomatosis of the parathyroid glands, with the adrenal glands small, weighing only 5 Gm., but otherwise looking normal. The uterus and the ovaries were likewise atrophic. In rare cases of pluriglandular insufficiency, plain

63. Silver, S.: Arch. Int. Med. **51**:174, 1933.

64. Rose, E., and Weinstein, G.: Endocrinology **20**: 149, 1936.

65. Gilford, H.: The Disorders of Postnatal Growth and Development, London, Adlard and Son, 1911.

66. Hutchinson, J.: Med.-Chir. Tr., London **69**:473, 1886.

67. Claude and Gougerot: J. de physiol. et de path. gén. **10**:468, 1908.

68. Castleman, B., and Hertz, S.: Arch. Path. **27**:69, 1939.

atrophy, without proliferation of fibrous tissue, may be seen involving the same endocrine glands as in multiple endocrine sclerosis (Lindemann [69]).

Summarizing, one may say that only in cases in which evidence can be presented that the process consisting of sclerotic or simple atrophy has started simultaneously in multiple endocrine glands is the diagnosis of multiple endocrine sclerosis or atrophy justified.

As to the cause of the disease, some investigators believe that it is due to a chronic infection of unknown cause; syphilis as an etiologic factor also has been mentioned. In addition, one has also to consider congenital weakness or inferiority of the endocrine system, which, unable to stand physiologic strain, gradually breaks down and offers a picture of atrophy with or without subsequent fibrosis.

Clinically, anorexia nervosa easily can be confused with hypophysial cachexia, but as far as is known in the former, which might have a psychiatric rather than an endocrine background, there is no obvious pathologic condition of the hypophysis. Richardson,[70] who had occasion to examine 6 cases of anorexia nervosa, observed in a case that ended fatally that the hypophysis was normal. Likewise, in a typical case studied by Brosin and Apfelbach [71] the hypophysis was found without pathologic change. (See also Magendantz and Proger.[72])

III

CUSHING'S DISEASE (PITUITARY BASOPHILISM)

More than one decade ago Cushing [73] described a syndrome which, though frequently observed and described by previous authors, was not recognized before as an independent clinical and pathologic entity. On the basis of 12 cases, many of which were collected from the literature, Cushing established a syndrome that is characterized by rapidly increasing, often painful obesity, osteoporosis with a tendency toward kyphosis of the thoracic portion of the spinal column, spontaneous fractures of bones, sexual dystrophy, hypertrichosis and purplish striae particularly on the abdomen.

Whereas Cushing interpreted the new syndrome as due to basophilic hyperpituitarism, some authors have questioned its primary hypophysial origin, while others regard the syndrome as due to a special form of hypopituitarism. The syndrome, which is more frequently observed in women than in men, is frequently accompanied by arterial hypertension, glycosuria, erythemia, leukocytosis, extreme dryness and pigmentation of the skin, acrocyanosis and purpura-like hemorrhages. The sexual dystrophy leads in males to impotence and in females to amenorrhea and sterility. Subjective disturbances are pain in the back and the abdomen, fatigue and weakness. Polyphagia, polydipsia, polyuria and anasarca have been seen in some cases. In rare instances the disease involves children.[74] Not always does the patient show all of these symptoms. The obesity, which in a characteristic manner alters the face, the neck and the trunk but not the extremities, is always present together with the hypertrichosis, the kyphosis and the purple striae on the greatly protruberant abdomen. These symptoms confer on the patient a characteristic appearance, so that when the condition is fully developed, the right diagnosis is hardly ever missed.

The pathologic observations include hyperplasia of the adrenal cortex, basophilic adenoma of the hypophysis in the large majority of cases and Crooke's "hyaline change" of the basophilic cells. Hyperplasia of the basophilic cells in the form of numerous ill defined microscopic nodules (Rutishauser [74a]) may be seen instead of adenoma. Also observed are marked lipomatosis of the parathyroid glands and atrophy of the thyroid gland, as well as a diffuse or nodular colloid goiter, luteinization of graafian follicles in the ovary, atrophy of the testicles with decrease of spermatogenesis and disappearance of Leydig's interstitial cells, osteoporosis, kyphosis, spontaneous fractures, arteriosclerosis and central or diffuse infiltration of the liver.

According to Cushing, the basophilic adenoma of the hypophysis represents the cause of the disease, while the changes of the adrenal glands and the gonads might be secondary in nature. The obesity is supposed to be due to a disturbance in the tuberohypophysial mechanism, the osteoporosis to the lesion of the parathyroid glands and, finally, the arterial hypertension to the basophilic invasion of the posterior lobe of the hypophysis. To this conception Kraus [75] objected, first of all, by pointing to those cases of Cushing's disease in which, despite thorough

69. Lindemann, E.: Virchows Arch. f. path. Anat. **240**:11, 1923.

70. Richardson, H. B.: Arch. Int. Med. **63**:1, 1939.

71. Brosin, H. W., and Apfelbach, C.: J. Clin. Endocrinol. **1**:272, 1941.

72. Magendantz, H., and Proger, S.: J. A. M. A. **114**:1973, 1940.

73. Cushing, H.: Arch. Int. Med. **51**:487, 1933.

74. (*a*) Rutishauser: Deutsches Arch. f. klin. Med. **175**:640, 1933. (*b*) Jamin, F.: München. med. Wchnschr. **81**:1045, 1934. (*c*) Farber, J. E.; Gustina, F. J., and Postoloff, A. V.: Am. J. Dis. Child. **65**:593, 1943.

75. Kraus, E. J.: (*a*) Klin. Wchnschr. **13**:487, 1934; (*b*) **16**:533, 1937.

examination, no basophilic adenoma or hyperplasia was found.[76] As another argument against Cushing's conception that the basophilic adenoma causes the syndrome, Kraus pointed out that the tiny size of the adenoma in some of the cases contradicts the long duration of the disease. Diameters of 1.5 and 2.5 mm. were recorded in cases which had lasted five years. One of these small tumors was observed by Cushing himself.

Another factor that makes Cushing's theory untenable is the fact that often a much larger basophilic adenoma does not produce the disease despite the presence of well built, highly differentiated basophilic cells in it (several cases published by Jedlicka[77]). Susman[78] examined the hypophysis in 260 routine necropsies and found basophilic adenoma in 3 per cent, which in his opinion might cast doubt on the association of a syndrome such as "pituitary basophilism" with basophilic adenoma. And finally, in some cases of Cushing's disease the adenoma found in the hypophysis was not basophilic in nature, as in Fuller's[79] case, in which a chromophobe adenoma was associated with the disease. In another case, reported by Salus,[80] that of a 30 year old woman with several characteristic symptoms of Cushing's disease, chromophobe carcinoma was found. The patient, after ten years of suffering from headaches, amenorrhea and obesity with weight increasing from 60 to 90 Kg., suddenly started losing weight, and signs of diabetes insipidus, striae, hypertrichosis and osteoporosis developed. The autopsy revealed a huge nodular tumor of medullary character, as large as a hen's egg, with extensive destruction of bone and invasion of the epipharynx. The tumor was made up of small chromophobe cells with no granulation demonstrable by specific staining. The hypophysis itself could not be found, having been apparently destroyed by the tumor. Just as in other cases bearing the features of Cushing's disease, the adrenal glands were greatly enlarged, weighing 21 Gm. and laden with lipoid, an observation that illustrates the significance of the adrenal glands in the genesis of the disease.

Kraus is inclined to believe that the basophilic adenoma may represent a secondary feature, perhaps compensatory in nature, comparable to the parathyroid adenoma found in association with osteomalacia. The aforementioned facts certainly suggest that the basophilic adenoma is not as essential in the genesis of the disease as first was thought. On the contrary, as Heinbecker[81] recently pointed out, it seems to be more probable that one has to deal in Cushing's disease with a condition of underactivity rather than overactivity of the gland.

Besides the basophilic adenoma there is another lesion in the hypophysis, the so-called hyaline change of the basophilic cells, described by Crooke[82] and called by him an alteration of "fundamental significance" for the development of the disease. Excellent pictures illustrate the lesion, which is characterized by dense homogeneous hyaline cytoplasm replacing the basophilic granulation. This lesion is found in but few conditions other than Cushing's disease and then merely to a slight degree. Ecker[83] saw it in 1.1 per cent of cases other than those of Cushing's disease. In addition to the hyalinization of the cytoplasm, the basophilic cells show, as essential changes, ballooning of their nuclei, excessive vacuolation and a tendency toward multinucleation and cell enlargement. Only a few authors were unable to find Crooke's change of the basophilic cells. Among them were Hall and Kellett.[76f] In their case the basophilic adenoma was also unobserved. Eisenhardt and Thompson,[84] who reviewed 63 cases of Cushing's disease, found true basophilic adenoma in 33, chromophobe adenoma in 3, mixed cell adenoma in 2 and a cancerous tumor with metastasis in 1; 1 case remained unclassified. Crooke's change was found in all these instances. Farber and coworkers[74c] in their case did not observe ripe basophilic cells or basophilic adenoma.

In contrast to the basophilic adenoma, which in many instances was absent, the hyperplasia of the adrenal glands seems to be fairly constant and may reach a remarkable degree, as in a case recently reported by Paschkis and associates,[84a] in which the adrenal glands weighed 60 Gm., compared with the normal weight of 7 to 11 Gm. In some cases of Cushing's disease, tumor of the adrenal cortex was encountered and no adenoma

76. (*a*) McCallum, W. G.; Futcher, T. B.; Duff, G. L., and Ellsworth, R.: Bull. Johns Hopkins Hosp. **56**:350, 1935. (*b*) Pons, J. A., and Pappenheimer, A. M.: Puerto Rico J. Pub. Health & Trop. Med. **13**:115, 1937. (*c*) Leyton, cited by Kraus.[75b] (*d*) Gellerstedt, N., and Lundquist, R.: Upsala läkaref. förh. **45**:233, 1939. (*e*) Maranon, G.: Ann. d'endocrinol. **1**:241, 1939. (*f*) Hall, G.; Kellett, C. E., and Stephenson, G. E.: Lancet **2**:862, 1939 Kraus.[75b]

77. Jedlicka, V., cited by Kraus.[75b]

78. Susman, W.: Brit. J. Surg. **22**:539, 1935.

79. Fuller, C. J.: Lancet **2**:181, 1936.

80. Salus, F.: Ztschr. f. d. ges. Neurol. u. Psychiat. **148**:574, 1933.

81. Heinbecker, P.: Medicine **23**:225, 1944.

82. Crooke, H. C.: J. Path. & Bact. **41**:339, 1935.

83. Ecker, A. D.: Endocrinology **23**:609, 1938.

84. Eisenhardt, L., and Thompson, K. W.: Yale J. Biol. & Med. **11**:507, 1939.

84a. Paschkis, P. A.; Herbut, P. A.; Rakoff, A. E., and Cantarow, A.: J. Clin. Endocrinol. **3**:212, 1943.

of the hypophysis (Calder and Porro,[85] Humphreys[86] and others). This caused several authors to interpret the disease as "hyperadrenocorticism" (Albright, Parson and Bloomberg,[87] Bauer,[88] Maranon[76a] and others). In connection with this problem it might be of interest that in a case of Crile[89] enervation of the adrenal glands resulted in the disappearance of the syndrome despite the fact that, as later was shown at autopsy, a basophilic adenoma was present in the hypophysis. Graef and associates[90] described a case in which they found a metastasizing carcinoma of the adrenal cortex, a tiny adenoma of the anterior lobe and Crooke's hyaline change. A quite similar case was reported by Ravid.[91] It is of interest that Crooke himself found the hyaline change not only in neoplasms of the adrenal cortex but also in carcinoma of the thymus; just as Norris[92] observed it in arrhenoblastoma. It seems that the hyaline change of the basophilic cells in Cushing's disease represents an injury resulting from hyperfunction of the adrenal cortex, which in the majority of the cases, though not in all, is followed by regeneration and proliferation of the basophilic cells, usually in the form of a small basophilic nodule or more rarely in the form of diffuse or nodular hyperplasia (Kraus[93]; Heinbecker[81]). This conception seems to explain best the genesis of the two hypophysial changes in Cushing's disease: Crooke's hyaline change of the basophilic cell, representing a retrogressive process, and the basophilic adenoma, a progressive and possibly reparatory process.

According to an experiment by Kraus,[94] the basophilic cells forming the adenoma in Cushing's disease seem to lack the ability to produce gonadotropic hormone, since parts of a basophilic adenoma from a patient with Cushing's disease when implanted into infantile female mice did not stimulate follicular growth, nor any luteinization of follicles, whereas the same experiment with parts of a basophilic adenoma from a patient who did not have Cushing's disease resulted in a strongly positive "pregnancy reaction." More research, however, is required before the result of this single experiment may be generalized.

Many cases of endocrine disease have been described showing certain features of Cushing's disease without matching exactly the picture of this disease. The changes in the hypophysis were various. Basophilic adenoma, other forms of adenoma, eosinophilia of the hypophysis, edema, adenomatous or diffuse hyperplasia and hypernephroma of the adrenal cortex were observed in those cases, some of which bore the feature of adipositas dolorosa. In atypical cases the separation of Cushing's disease from interrenalism may cause difficulties. The typical lesions of bone, the characteristic distribution of the fat, the purple striae and the much lesser hirsutism and virilism will help to differentiate the syndrome of Cushing from the classic type of interrenalism.

Achard and Thiers[95] described a syndrome that they called *diabète des femmes à barbe* (diabetes in bearded women). Considering the essential symptoms, namely, the masculine hairiness of the face, the disorder of the sexual functions, the obesity involving the trunk but not the extremities, the striae, the hypertension and the glycosuria, one can be in no doubt that the syndrome is identical with Cushing's disease. In 1 case the autopsy revealed large adrenal glands, a colloid goiter and fibrotic ovaries. In other cases obesity, hypertrichosis and osteoporosis were noted together with diabetes mellitus.[96]

85. Calder, R. M., and Porro, F. W.: Bull. Johns Hopkins Hosp. **57**:99, 1935.
86. Humphreys, E. M.: Am. J. Path. **18**:764, 1942.
87. Albright, F.; Parson, W., and Bloomberg, E.: J. Clin. Endocrinol. **1**:375, 1941.
88. Bauer, J.: Schweiz. med. Wchnschr. **39**:938, 1936.
89. Crile, G., cited by Kraus[75b] and by Kessel, F. K.: Ergebn. d. inn. Med. u. Kinderh. **50**:620, 1936.
90. Graef, T.; Bunim, T. T., and Rottino, A.: Arch. Int. Med. **57**:1085, 1936.
91. Ravid, J. M.: Am. J. Path. **18**:764, 1942.
92. Norris, E. H.: Am. J. Cancer **32**:1, 1938.
93. Kraus, E. J., in Veit, J.: Handbuch der Gynäkologie, Berlin, Julius Springer, 1936, vol. 9, p. 580.
94. Kraus, E. J.: Klin. Wchnschr. **11**:1020, 1932.
95. Achard, C., and Thiers, J.: Bull. Acad. de méd., Paris **86**:51, 1921.
96. Weber, F. P.: Brit. J. Dermat. **38**:1, 1926. Brown, W. L.: Am. Med. **23**:53, 1928. Weil, P. E., and Plichet: Bull. et mém. Soc. méd. d. hôp. de Paris **45**:312, 1921. Shepardson, H. D., and Shapiro, E.: Endocrinology **24**:237, 1939.

Book Reviews

Virus as Organism: Evolutionary and Ecological Aspects of Some Human Virus Diseases. Frank MacFarlane Burnet, M.D., F.R.S., director, Walter and Eliza Hall Institute of Research in Pathology and Medicine, Melbourne, Australia. Pp. 134. Price $2. Cambridge, Mass.: Harvard University Press, 1945.

This monograph is an expansion of the Dunham Lectures for the Promotion of Medical Sciences, Harvard University, 1944. The human virus diseases subjected to analysis from the biologic point of view are: herpes simplex; poliomyelitis; psittacosis and related infections; smallpox, alastrim and vaccinia; yellow fever, and influenza. Special sections are devoted to the consideration of viruses (their reproduction, variation and survival), to the evolution and change in virus infection and to the reaction of the host to such infection. The evolutionary aspects of virus infection are discussed ably and instructively from factual as well as speculative angles. The book will interest greatly those who are concerned with virus diseases. The final paragraph presents a comprehensive perspective: "Big future developments in the finer laboratory study of viruses can be looked forward to with certainty. With further elucidation of their chemical structure, of their mode of using the enzyme systems of the host, and of the physical chemistry of the replication process, new conceptions of the relationship of viruses to other biological units may arise. For some purposes it may be desirable to allocate them to a new category sharply differentiated from all other living organisms, but as far as the doctor, the public health administrator, and the biological experimenter are concerned, the pragmatic necessity will remain that virus be regarded as organism—self-reproducing, varying and surviving like any other living being."

Correspondence

INCIDENCE OF ESOPHAGEAL CARCINOMA IN THE WEST INDIES

To the Editor:—In an article, "Esophageal Carcinoma in British West Indian and Panamanian Negroes," which appeared in the ARCHIVES OF PATHOLOGY (**39**: 79, 1945), Tomlinson and Wilson came to the conclusion that this type of cancer ranks third as to number of cases among all the types of carcinoma observed at autopsy in their material. This is at variance with recent reports suggesting that esophageal cancer is far more frequent in white males.

In their references to the literature they omit mention of the fact that in my paper "The Incidence of Malignant Tumors in Unselected Autopsy Material at Curaçao, Netherlands West Indies," which appeared in the *American Journal of Cancer* (**40**:355, 1940), it was pointed out that, of 60 cancerous *epithelial* tumors found in 650 autopsies performed on Negroes from Curaçao, 15, or 25 per cent, were esophageal cancers, this type of tumor ranking first and carcinoma of the stomach ranking second.

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